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Prenatal and early postnatal long-chain polyunsaturated fatty acid status

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Bouwstra, H. (2007). *Prenatal and early postnatal long-chain polyunsaturated fatty acid status: do they affect neurodevelopmental outcome in healthy term infants?* s.n.

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Prenatal and early postnatal long-chain polyunsaturated fatty acid status

Do they affect neurodevelopmental outcome in healthy term infants?

Hylco Bouwstra

“Verwondering is een authentiek menselijke eigenschap, die bij volwassenen helaas bedreigd wordt door ‘gezond realisme’. In de realistische maatschappij van volwassenen is verwondering meer en meer gereduceerd geraakt tot het terrein van de kunsten en (soms) van de wetenschap.”

Nico Smit, lector Hogeschool van beeldende kunsten, muziek en dans te Den Haag

RIJKSUNIVERSITEIT GRONINGEN

Prenatal and early postnatal long-chain polyunsaturated fatty acid status

Do they affect neurodevelopmental outcome in healthy term infants?

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, prof. dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 3 oktober 2007
om 16:15 uur

door

Hylco Bouwstra

geboren op 30 juli 1980

te Heerenveen

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Prenatal and early postnatal long-chain polyunsaturated fatty acid status. Do they affect neurodevelopmental outcome in healthy term infants?
Thesis University Medical Center Groningen. Summary in Dutch.
ISBN no: 9789036730389

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The publication of this thesis was financially supported by Numico Research B.V.
The support is gratefully acknowledged.

Lay-out: H. Bouwstra
Cover: illustration by Luna Tjeerdsma, 2006
Printed by Gildeprint Drukkerijen, Enschede, The Netherlands

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Foreword

This thesis describes the main findings of the longitudinal LCP-project that was started in 1997. The LCP-project is an international multidisciplinary collaboration between the departments of Neurology and Pathology and Laboratory Medicine of the University Medical Center Groningen, the department of Pediatrics of the University of Pécs, Hungary, and Numico Research Germany, Friederichsdorf, Germany. Numico Research sponsored the project. Initially the project leaders were prof. dr. E.R. Boersma, and prof. dr. M. Hadders-Algra, later on the role of prof Boersma was taken over by prof. dr. F.A.J. Muskiet. Between 1997 – 2002 two investigators, Mrs. J.A.L. Wildeman and Mrs H.M. Tjoonk have investigated 472 enrolled infants and gathered all infant follow-up data of the LCP-project. They started data-analysis, but at the end of their employment period, major part of the data-analyses still had to be carried out. At the end of 2002 the author of this thesis took part in the analyses of the follow-up data of the project as a medical student which ultimately resulted in the writing of the present thesis. The MD/PhD program of the 'Junior Scientific Masterclass' enabled him to complete his PhD thesis. A major aim of the LCP-project was to evaluate the effects of long-chain polyunsaturated fatty acid (LCPUFA) supplementation on neurodevelopment of healthy term infants. A secondary objective was to compare the developmental outcome of formula fed infants with breastfed infants. In addition, the prenatal fatty acid status measured at birth was correlated with the neurodevelopmental outcome at 3 and 18 months.

Abbreviations

AA	arachidonic acid
ALA	alpha-linolenic acid
DHA	docosahexaenoic acid
EFA	essential fatty acids
GMs	general movements
OOS	obstetrical optimality score
PDI	psychomotor developmental index
LCPUFA	long-chain polyunsaturated fatty acids
MND	minor neurological dysfunction
MDI	mental developmental index
MUFA	monounsaturated fatty acids
NOS	neurological optimality score

General Introduction

1

1 General introduction

1.1 Essential fatty acid status

1.1.1 Introduction

The importance of essential fatty acids and long-chain polyunsaturated fatty acids (LCPUFAs) in human development finds its origin in 1929 when Burr and Burr demonstrated the essentiality of linoleic acid[1]. They demonstrated that the removal of linoleic acid from the diet leads to symptoms of deficiency in laboratory animals, such as dermatitis, growth retardation and infertility[1]. These findings inspired Hansen and co-workers to study fatty acid deficiency in humans[2]. The discovery of the essentiality of omega-3 fatty acids in infant nutrition was not acknowledged until Holman's demonstration in 1982 that lack of omega-3 fatty acids was associated with clinical abnormalities, including paresthesia, weakness, inability to walk and impaired vision in a 6-year-old child maintained on total parenteral nutrition[3]. Meanwhile, evidence was accumulating that omega-3 deficient diets induced visual abnormalities in subhuman primates[4]. Since then considerable progress has been made in the understanding of the physiological functions of essential fatty acids in animals and humans and their role in chronic diseases. The role of essential fatty acids in the development of the human central nervous system will be the focus of this thesis. First, a general overview will be given on essential fatty acids. Next, the effects of LCPUFA supplementation during early human development will be reviewed.

1.1.2 Nomenclature of fatty acids

Fatty acids consist of a long linear hydrocarbon chain with a carboxylic acid group. Naturally occurring fatty acids contain even numbers of carbon atoms in straight chains that vary in length from 6 to 26 carbon atoms. By convention, the carbon-atoms are numbered from the carboxylic acid end onwards (Figure 1). The double bonds in the naturally occurring fatty acids in humans are methylene-interrupted. Almost all naturally occurring double bonds have a *cis* configuration, which indicates that the two hydrogen atoms adjacent to the double bond point to the same side of the molecule as opposed to the *trans* configuration. Apart from the systematic and common (trivial) names of fatty acids, a shorthand notation can be used. The first number is the number of carbon atoms in the molecule. The second number, after the colon, is the number of double bonds. The last number indicates the number of methylene carbons form the methyl carbon end (written as 'n minus' or ω) to the nearest double bond. See figure 1, last collumn. Unsaturated fatty acids can be divided in four families according to the position of the first double bond: *n*-9, *n*-7, *n*-6, and *n-3*. This thesis focuses primarily on polyunsaturated fatty acids of the *n*-6 and *n*-3 families. By definition, polyunsaturated fatty acids have two or more double bonds. LCPUFAs of the *n*-3 and *n*-6 families consist of 20 or more carbon atoms and have two or more double bonds. Consequently, linoleic and α-linolenic acids are excluded when using the term LCPUFA (Table 1). Arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3) are the most abundantly present LCPUFAs in the central nervous system. A short list of the most relevant fatty acids of this thesis is presented in table 1.

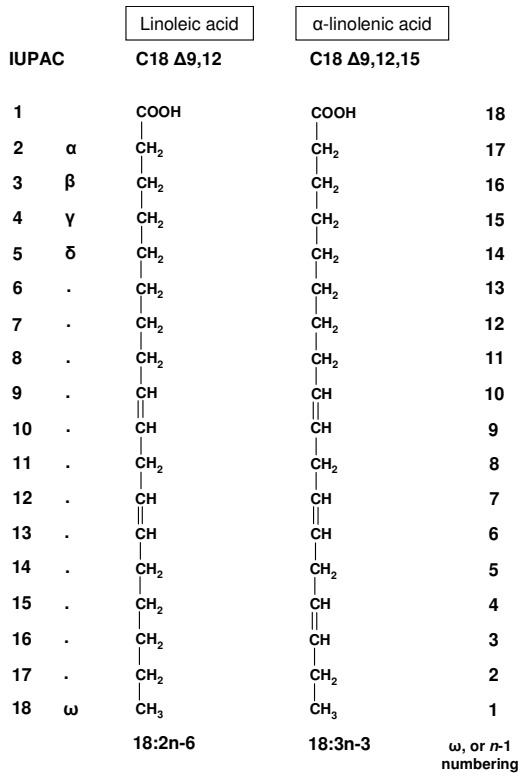

FIGURE 1. Naming and molecular structure of fatty acids.

TABLE 1. List of the most relevant fatty acids of this thesis

Shorthand notation	Trivial name
16:0	palmitic acid
18:0	stearic acid
18:1n-9	oleic acid
18:2n-6	linoleic acid
18:3n-3	α-linolenic acid
20:3n-9	mead acid
20:4n-6	arachidonic acid (AA)
22:6n-3	docosahexaenoic acid (DHA)

1.1.3 Essentiality of linoleic and α-linolenic acid

Vertebrate animals and humans cannot synthesize essential fatty acids by definition. Removal of essential fatty acids from the diet leads to symptoms of deficiency[5-7]. Linoleic (18:2n-6) and α-linolenic acid (18:3n-3) are the parent essential fatty acids. It is important to note that the parent essential fatty acids can be converted into LCPUFAs by humans, most animals, some algae, bacteria and fungi, but not in plants[8]. To summarize, the essentiality of nutritional components indicates the distinction between nutrients which can be endogenously synthesized and which cannot be synthesized and consequently must be externally supplied.

1.1.4 Metabolism of essential fatty acids

From the parent essential fatty acids, two families (*n*-3 and *n*-6 series) of polyunsaturated fatty acids can be synthesized. Alpha-linoleic acid (ALA) is the precursor of *n*-3 polyunsaturated fatty acids such as DHA. Linoleic acid (LA) is the precursor of *n*-6 polyunsaturated fatty acids such as arachidonic acid (AA). There is no interchange between *n*-3, *n*-6 and *n*-9 fatty acids possible. Figure 2 shows a schematic overview of the conversion of essential fatty acids into polyunsaturated fatty acids by various enzymes. The parent essential fatty acids are transformed into LCPUFAs by enzymes, which subsequently add two carbon units and insert double bonds into molecules. Three different desaturases are present in human tissue which can introduce double bonds at specific places in fatty acid molecules[9]. The desaturases are embedded in the membranes in an interconnected network of tubules, vesicles and sacs of the endoplasmic reticulum. These enzymes are highly preserved across kingdoms of species to provide unsaturated fatty acids for the synthesis of lipid membranes. Stearoyl CoA desaturases (SCD or Δ9-desaturase) catalyse the synthesis of monounsaturated fatty acids. As the name implies this enzyme introduces a double bond at the ninth position from the carboxyl end of fatty acids to produce the n-9 series of unsaturated fatty acids. Delta-5 and delta-6-desaturases catalyse the synthesis of LCPUFAs and are widely expressed in human tissues, especially in the liver[10,11]. To synthesize DHA, one last step is necessary in the peroxisome for a β-oxidation reaction. The synthesis of DHA from the precursor α-linolenic acid is variable and inefficient (less than 4%)[12]. The inefficient synthesis of DHA combined with the low intake of DHA and the high intake of linoleic acid (see below) in the Western diet provides the rationale to investigate the consequence of marginal DHA deficiency for brain development and health[13,14].

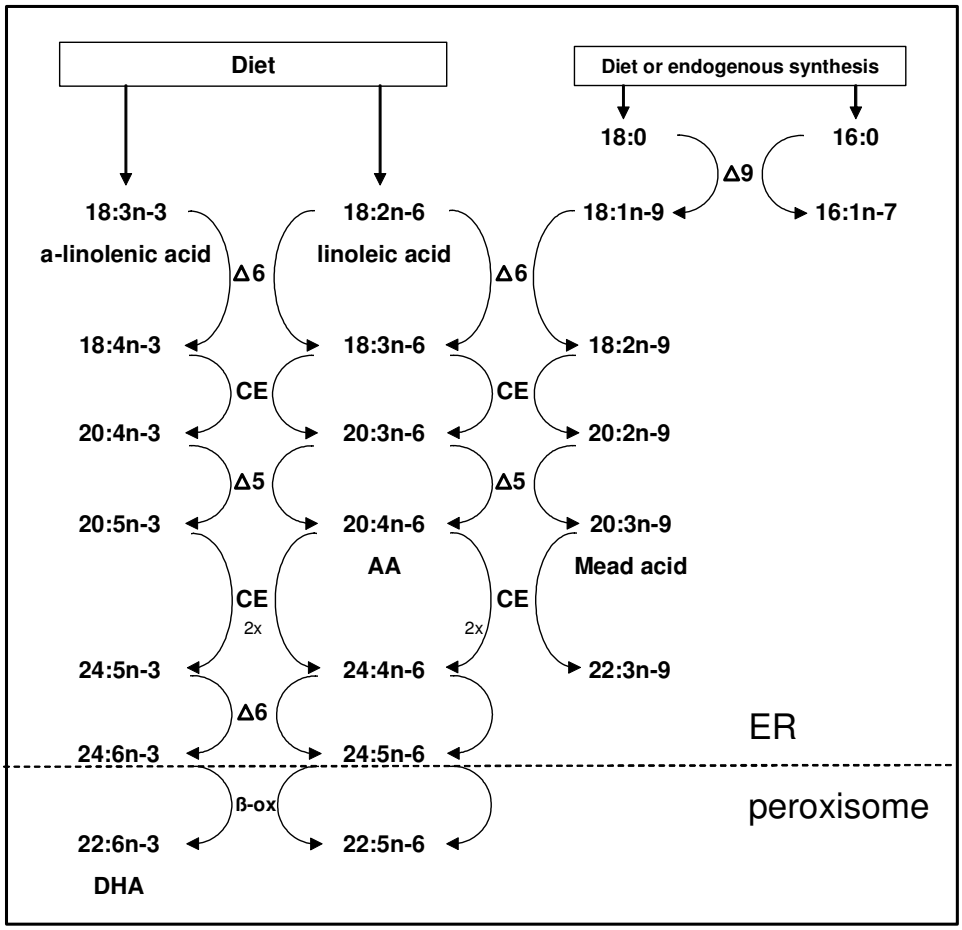

FIGURE 2. Schematic overview of conversion of essential fatty acids into polyunsaturated fatty acids. In addition, the conversion of dietary n-7 and n-9 fatty acids is shown.

1.1.5 Regulation of LCPUFA metabolism

The total LCPUFA content such as in the brain and retina is relatively constant because of feedback regulation mechanisms. The rate-limiting step in LCPUFA synthesis is the desaturation by Δ6-desaturase which activity is negatively regulated by polyunsaturated fatty fatty acid end products (Figure 2)[15]. Also the Δ5 enzyme is suppressed when sufficient polyunsaturated end products are available[10,11,16]. This negative feedback mechanism helps to maintain LCPUFA levels. Deficiency of solely *n*-3 polyunsaturated fatty acids is compensated by an increase in 22:5n-6 synthesis as has been demonstrated in young adult rats and baboons[17,18]. Makrides et al. found an inverse relationship between 22:5n-6 and DHA status in the human cortex[19]. Furthermore, the *n*-3 and *n*-6 polyunsaturated fatty acids inhibit Δ9-desaturase, while the endproduct 18:1n-9 has no suppressing effect on Δ9-desaturase[20]. Limited availability of both *n*-3 and *n*-6 polyunsaturated fatty acids leads to more synthesis of n-9 fatty acids which partially could compensate for a low polyunsaturated fatty acid status. Delta-6-desaturase has an increasing preference for its substrates in the order α-linolenic acid, linoleic acid, oleic acid (18:1n-9). Consequently one particular member of the n-9 fatty acids named mead acid (20:3n-9) has an inverse relationship with EFA status and can be used as a marker for EFA deficiency[21,22]. The ratio between 22:5n-6 (docosapentaenoic acid; DPA) and DHA can be used as a marker for DHA deficiency[22].

1.1.6 Inverse relationship between *trans* fatty acids and LCPUFAs

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. The properties of *trans* fatty acids resemble those of saturated fatty acids due to the more rigid molecule than *cis*-unsaturated fatty acids. The most important source of *trans* fatty acids in humans is external supply via the diet, because no endogenous synthesis of *trans* fatty acids is possible. *Trans* fatty acids originate mainly from industrial hydrogenated fatty acids which e.g. can be found in margarines, baked goods and fast foods (80-90% of intake) and from dairy products which contain by-products of ruminating bacteria (2-8% of intake)[23]. *Trans* fatty acids cross the placenta, and maternal milk reflects the daily intake of *trans* fatty acids[23]. *Trans* fatty acids are incorporated in body tissues, but not in the central nervous system. Umbilical and blood lipid *trans* fatty acids are inversely related with LCPUFA status[23,24], which has also been demonstrated in our study population[25]. A partially inhibitory effect of *trans* fatty acids on the Δ-6 fatty acid desaturase activity has been found in pregnant rats fed high amounts of *trans* fatty acids[26]. In preterm and term infants *trans* fatty acids are inversely correlated to infantile birth weight[27,28]. However, multigenerational animal studies do not demonstrate any detrimental effect of high *trans* fatty acid intake on weight, growth and longlevity[23]. This does however, not preclude subtle negative effects of *trans* fatty acids on brain development of human infants. At present, it is unclear whether *trans* fatty acids, apart from the well established relationship with coronary heart disease[29], exert also negative effects on the neurodevelopmental outcome of healthy term infants. We therefore measured the umbilical *trans* fatty acid status at birth in a subpopulation of the LCP-project which enables us to study the relationships between prenatal *trans* fatty acids and neurodevelopmental outcome at birth and at the follow-up ages of 3 and 18 months.

1.1.7 Regulation of LCPUFAs by transcription factors

Another important mechanism for the regulation of LCPUFA-metabolism is the induction of desaturases by various transcription factors. Some transcription factors cause an increase in enzyme synthesis by preventing the repression of the gene responsible for enzyme synthesis at the level of the promoter region. Two transcription factors play a key role in the regulation of desaturases: sterol regulatory element binding protein (SREBP) and peroxisome proliferators activated receptor-α (PPARα). In turn, highly complex mechanisms regulate the transcription of those two transcription factors (see review of Nakamura & Nara 2004[30]). In short, an isoform of SREBP-1c activates target genes involved in fatty acid synthesis. Polyunsaturated fatty acids suppress SREBP-1c. Because SREBP-1c is also involved in monounsaturated fatty acid synthesis, it has been hypothesized that SREBP-1c maintains the total unsaturated fatty content in cell-membranes. PPARα is a transcription factor that is believed to induce fatty acid oxidation. Polyunsaturated fatty acids, including LCPUFAs activate PPARα and thereby stimulate fatty acid oxidation. In summary, the two mentioned SREBP-1c and PPARα transcription factors are somesort of sensors of LCPUFA-status and induce fatty acid synthesis and fatty acid oxidation, respectively, to regulate the unsaturated fatty acid content in the cell.

1.1.8 Biochemical functions of LCPUFAs

An important function of fatty acids is deposition of energy that in turn can be used for cellular processes by means of β-oxidation. Fatty acids are stored in adipose tissue as triacylglycerols. Triacylglycerol molecules consist of the trihydric alcohol glycerol esterified with three fatty acid molecules. In adipose tissue, twelve till sixteen percent of fatty acids consist of linoleic acid, whereas the storage capacity of α-linolenic acid is limited (≈1%)[31]. Only small amounts of AA are present in adipose tissue. In addition, essential fatty acids are used as an energy source. Eighteen carbon polyunsaturated fatty acids are even preferentially oxidized for energy. For instance, it has been estimated that linoleic acid is oxidized about 50% faster than palmitic acid (16:0) and three times faster than stearic acid (18:0). Alpha-linolenic acid is oxidized even more than linoleic acid[32]. These findings suggest that humans have evolved in an environment with abundant sources of essential fatty acids. As described in detail in the previous section on LCPUFA metabolism, *n*-3 and *n*-6 polyunsaturated fatty acids are not regulated independently, which suggests that humans have evolved in an environment with a balanced ratio of *n*-3 and *n*-6 polyunsaturated fatty acids. An alternative explanation may be that most symptoms of *n*-3 polyunsaturated fatty acid deficiency which affect survival, can be alleviated by *n*-6 polyunsaturated fatty acid intake alone. See section 1.2.2 for more details concerning the role of the ancient diet on present day dietary requirements.

Fatty acids are also used for the formation of membrane lipids. Especially DHA and AA become incorporated into membranes. Cell membranes predominantly consist of phospholipids (around 75%), sphingolipids and plasmalogens which have a hydrophilic head and a hydrophobic tail. The chemical structure of a phospholipid is depicted in figure 3. A phospholipid consists of a glycerol part of which the three adjacent carbons are numbered *sn*-1, *sn*-2, and *sn*-3 according to their stereospecific position. The *sn*-1 position is mostly occupied by saturated fatty acids, primarily stearic (18:0) and palmitic acids (16:0). The *sn*-2 is variably occupied by unsaturated fatty acids such as DHA and AA, depending on phospholipid class and cell type. The *sn*-3 position is occupied by a phosphate group with variable hydrophilic head groups that determine phospholipid class such as choline in phosphatidylcholine (PC). Phospholipids, which are highly unsaturated,

because of incorporation of LCPUFAs, can influence the biophysical properties of membranes. Especially DHA is readily incorporated into phospholipids. DHA-rich membranes affect acyl chain order and "fluidity", phase behaviour, elastic compressibility, permeability, fusion, flip-flop and protein activity in the cell membrane[33]. The exact interactions between DHA and specific cellular proteins are beginning to be unravelled[34]. Because of the overall effects of DHA on general biophysical properties, one can surmise that DHA has a general effect on a variety of cellular functions.

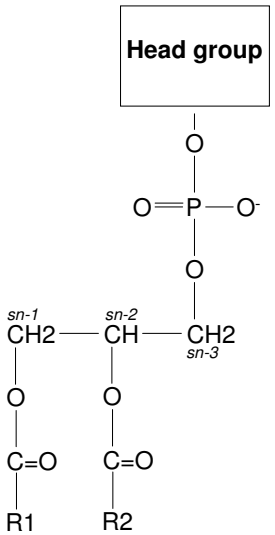

FIGURE 3. Chemical structure of a phospholipid with three adjacent carbons named *sn*-1, *sn*-2 and *sn-3*. Various head groups can be placed at the phosphate group at the *sn-3* position: choline in phoshatidylcholine (PC), serine in phoshatidylserine (PS), ethanolamine in phosphatidylethanolamine (PE) and inositol in phosphatidylinositol (PI). R1, R2 = hydrocarbon chain.

LCPUFAs do not only affect cell function by modulation of cell membrane properties, but also act as precursors of important autocrine and paracrine mediators (e.g. eicosanoids and resolvins) which possess anti-inflammatory and neuroprotective properties[35]. The relative proportions of *n*-3 polyunsaturated fatty acids and AA at the *sn*-2 position of phospholipids or diacylglycerol affect the balance of these many different mediators. Especially AA is the precursor for potent eicosanoids that play an eminent role in the inflammation response. This could also explain the relative absence of AA in triglycerides in adipose tissue because of the potential harmful effects of free AA when released from triglycerides by lipases.
LCPUFAs also are ligands for nuclear receptors such as PPARs and retinoid X receptor that regulate gene expression. LCPUFAs do not only affect expression of genes involved in the regulation of LCPUFA synthesis and oxidation, but also affect expression of many other genes that play a role in the development of the brain[36]. Furthermore, a recent study of Kawakita et al. 2006 demonstrated that DHA promotes neurogenesis in both vitro and in vivo[37]. These discoveries imply that DHA does not only affect membrane properties but also has an effect on gene expression.

The recent discovery of these mechanisms may enhance our understanding of the complex interactions between diet and the expression of the genome in the developing nervous system.

1.1.9 Transport of LCPUFAs

During intra uterine life, LCPUFAs are supplied to the fetus via the placenta. The LCPUFA supply is dependent on the maternal dietary intake, maternal adipose tissue stores, and fetal and maternal endogenous synthesis. Pregnant women may have an increased capacity to convert the parent essential fatty acids into LCPUFAs in order to meet the high LCPUFA requirements during gestation[38]. Crawford has coined the term 'biomagnification' to describe the observed progressive increase in DHA concentration in phospholipids from maternal blood, cord blood, fetal liver, to the fetal brain[39]. Both AA and DHA are preferentially transferred from the maternal to the fetal circulation, which could be explained by preferential binding of LCPUFAs to placental plasma membrane fatty acid binding protein (p-$FABP_{pm}$)[40]. During prenatal life the fetus accumulates DHA in the liver and adipose tissues which act as a reservoir during early postnatal life. After birth, breastfeeding serves as an external dietary source of LCPUFAs. The LCPUFA content in human milk is highly influenced by maternal diet[41,42]. About 30% of the milk fatty acids derive directly from dietary intake of the mother. The other 70% derive directly from adipose tissue stores[40]. To summarize, during gestation LCPUFAs are preferentially transported to fetal tissues. The maternal LCPUFA supply to the fetus is at least partially dependent on maternal dietary intake of LCPUFAs. Maternal dietary intake also influences the LCPUFA supply to the breastfed infant during lactation.

1.1.10 Endogenous synthesis of LCPUFAs

Human fetus and neonates are able to convert linoleic and α-linolenic acids into AA and DHA, respectively. In other words, endogenous synthesis of LCPUFAs by means of the rate-limiting step of Δ-6 desaturase occurs in both preterm and term infants. As has been demonstrated in newborn rodents, the Δ-6 desaturase activity in the liver is relatively low at birth and high in the adult, whereas an opposite pattern exists of the Δ-6 desaturase activity of the brain[43,44]. It has also been demonstrated that the Δ-6 desaturase activityin the liver of human infants is also lower compared to adults[45]. The conversion of α-linolenic acid and the incorporation of DHA in the infant brain in this period of rapid brain growth is high[40]. Some studies indicate that endogenous synthesis in the preterm and term infant is not sufficient to meet the high requirements of LCPUFAs during the rapid brain growth shortly before and after birth [40,46]. In other words, LCPUFAs may be conditionally essential.

1.1.11 Dietary sources

Dietary sources of linoleic acid can be found in seeds of most plants, except for coconut, cocoa and palm vegetable oils[47]. Dietary sources of AA are meat, eggs and certain seafoods. Alpha-linolenic acid can be found in green leafy vegetables, nuts and some vegetable oils such as canola (rapeseed) and soybean oils. DHA and 20:5n-3 (EPA) are most abundant in fish and shellfish, particularly fatty fish such as salmon, tuna, mackerel, herring and sardines[47]. Table 2 shows the fatty acid composition of common nutrients[48]. Data are presented in g/100 g. The presented compositions can vary and represents only a gross indication of fatty acid compositions in every day used nutrients.

TABLE 2. Fatty acid composition of commonly used nutrients in g/100g[48]

	Total FA	Saturated FA	Mono-unsaturated FA	Polyunsaturated FA (*n*-6 + *n*-3)	*n*-3 FA		
Fats and oils					**ALA**	**EPA**	**DHA**
Olive oil	100	14	73	8	0.7	-	-
Sun flower oil	100	12	21	63	0.1	-	-
Soya bean oil	100	15	22	59	7.3	-	-
Rapeseed oil (canola oil)	100	7	59	29	10	-	-
Flax seed oil	100	10	16	65	55	-	-
Walnut oil	100	9	16	70	11.5	-	-
Safflower oil	100	10	12	74	0.1	-	-
Corn oil	100	14.5	30	51	0.9	-	-
Palm oil	100	48	37	10.5	0.3	-	-
Cocoa oil	100	87	6	1.5	-	-	-
Butter	82	51	24	2	≈ 1	-	-
Margarine (60% fat)	60	12	17	26	4	-	-
Fryer fat	100	46	27	8	≈ 1	-	-
Fryer oil	100	13	23	59	-	-	-
Mayonnaise	67	8	21	36	1-2	-	-
Meat							
Pork meat	30	10	12	4	<0.5	-	-
Lean pork meat	9	4	3.5	1	<0.5	-	-
Beef	15	6	7	1	<0.5	-	-
Lean beef	6	3	2	0.5	<0.5	-	-
Chicken fillet	4	1.5	1	1	<0.5	-	-
Fish oil							
Trout (raw)	5	1	2	2	0.1	0.2	0.8
Herring (raw)	13	4	6	2	0.2	0.5	0.7
Mackerel (raw)	16	3	8	3	0.2	0.7	1.1
Salmon	8	1.5	3	3	0.1	0.6	0.9
Fish oil (salmon)	100	19	29	40	1.0	13	18
Cod liver oil	100	22	46	22	0.9	7	11
Sardines (can)	16	4	4	7.5	0.4	0.9	0.8
Fish sticks	13	2.6	1.7	5.8	-	0.1	0.2
Nuts							
Walnuts	68	5	13	46	7	-	-
Peanuts	52	9.5	26	15	0.4	-	-
Hazelnuts	69	5	53	7	0.1	-	-
Cashew nuts	51	10	30	9	-	-	-
Pastiche nuts	48	6	33	7	-	-	-
Other							
Avocado	10	1.1	7.2	0.8	-	-	-
Olives	11	1.6	8.1	0.9	-	-	-

FA = fatty acids

1.1.12 Distribution of LCPUFAs in the central nervous system

LCPUFAs are not evenly incorporated into the various human tissues. The highest concentrations of DHA and AA are found in the nervous system and in the retina. Other polyunsaturated fatty acids, such as ALA, LA, EPA are only incorporated in small quantities in neuronal tissues. The human adult frontal cerebral cortex contains about 14 wt% DHA and 9.5 wt% AA[31]. Adipose tissue of humans living in Western societies does not contain large amounts of *n*-3 polyunsaturated fatty acids (≈ 1%)[31]. The small amounts of *n*-3 polyunsaturated fatty acids in adipose tissue derive from limited intake of DHA in the Western world. The storage capacity of DHA in adipose tissue is greater than 1% because Alaska natives who consume high amounts of *n*-3 fatty acids have higher amounts of DHA in adipose tissue[49]. A study that investigated the distribution of DHA and AA in

neonatal brain of breastfed baboons showed that DHA and AA is especially abundant in gray matter. The regions richest in DHA were all involved in motor function, i.e. the motor cortex (gyrus precentralis), basal ganglia and the superior colliculus. Noteworthy DHA content of the cerebellum, another structure that plays a large role in motor control, was relatively low[18]. The same study also investigated the effects of formula feeding with and without DHA/AA on the fatty acid composition of various brain regions. DHA levels in neonatal brains were lower in the non-supplemented formula group compared with the breastfed reference group. AA levels were relatively independent of AA in the diet. DHA levels in brains of DHA/AA supplemented baboons were the same as in brains of breastfed baboons except for the cerebral cortex, which showed lower DHA levels[18]. The aforementioned insights into the effects of LCPUFA supplementation on the topographical distribution of the increase in DHA composition of the non-human primate cerebrum could give valuable clues which neurological functions are involved in the observed beneficial effects of LCPUFA supplementation.

1.1.13 LCPUFA accretion and brain growth

The exponential increase in brain weight during the so-called brain growth spurt coincides with an increase in DHA content in the brain (Figure 4)[40]. Important ontogenetical events in brain development occur simultaneously during the brain growth spurt (third trimester to about 2 years after birth)[40,50]. During the third trimester and during the first year after birth axon and dendrite sprouting, axon elimination, synapse formation, myelination and glial cell proliferation take place after neuronal proliferation and migration have occured[51]. Since LCPUFAs have a general influence on the neuronal membranes and on neuronal gene transcription, it is possible that LCPUFA supply during pregnancy and after birth could have long-term effects on the neurodevelopment of children[33,36]. Active placental transfer of LCPUFAs ensures adequate supply of LCPUFAs during foetal development that is partly dependent on maternal dietary intake of LCPUFAs[52,53]. After birth, infants do not seem to synthesize sufficient amounts of LCPUFA from their precursors to cover their high needs because conversion of LCPUFAs from parent essential fatty acids is inefficient in humans (conversion rate of ALA into DHA is less than 1%). Red blood cell and plasma DHA concentrations decrease about 50% within 4 months after birth in the absence of dietary supply of DHA[31]. This drop in DHA content has also been demonstrated in the brain. Infants who received bottle-feeding without LCPUFAs had a 20% lower LCPUFA content in their brains compared to breastfed infants[19,46]. One can surmise that dietary supply of LCPUFAs during the growth spurt of the brain is essential for optimal brain development[50].

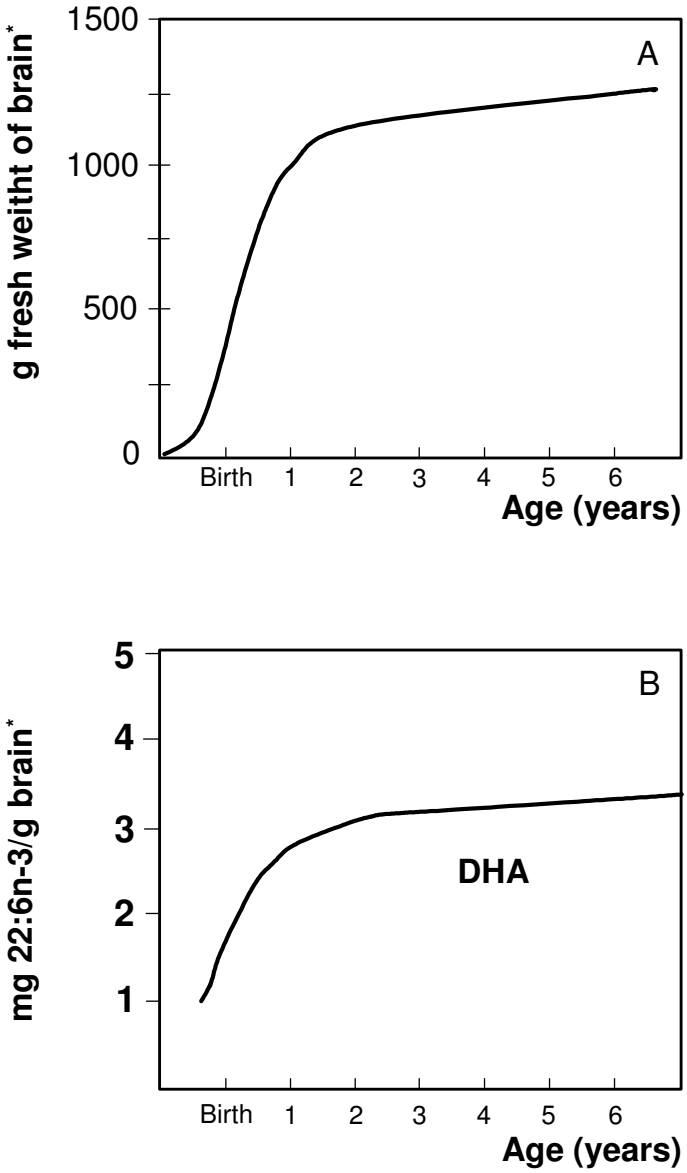

FIGURE 4

A. The increase of brain weight in grams shortly before and after birth.

B. Accretion of DHA in mg DHA/g brain according to the estimation of an increase in concentration of DHA of approximately 35 mg/wk which is in agreement with other estimates[40].

* Reprinted from "Lauritzen, Hansen, Jørgensen, Michaelsen. The essentiality of long chain *n-3* fatty acids in relation to development and function of the brain and retina. Progress in Lipid Research 40;1-94 2001, with permission form Elsevier".

1.2 Essential fatty acid deficiency

1.2.1 Clinical essential deficiency

Classical symptoms of essential fatty acid deficiency (dermatitis, growth retardation, infertility) can be reversed by *n*-6 fatty acids alone, whereas *n*-3 polyunsaturated fatty acids only partly can alleviate the deficiency symptoms[6]. Clinical essential deficiencies are extremely rare under normal physiological circumstances. Long-term parental nutrition without adequate amounts of essential fatty acid can cause clinical essential deficiency in humans[3]. Some of the symptoms of severe protein-energy malnutrition in the third world could be explained by essential fatty acid deficiency[54]. Important biological functions, that relate to the deficiency symptoms, are: (1) linoleic acid acts as an important constituent of the water barrier of the skin[40]; (2) arachidonic acid is a precursor of eicosanoids which are local hormones with a wide array of functions in physiological and inflammatory processes. Evidence of the essentiality of α-linolenic acid has been demonstrated in animals. Isolated α-linolenic acid deficiency induces minor cerebral dysfunctions as demonstrated in animals[55]. In 1982, Holman described a case of a 6-year-old child with α-linolenic acid deficiency involving neurological abnormalities caused by total parental nutrition rich in linoleic acid but low in α-linolenic acid[3]. The child experienced episodes of numbness, paresthesia, weakness, inability to walk, pain in the legs, and blurring of vision[3]. Other conditions in which clinical essential fatty acid deficiency is present are peroxismal metabolic disorders such as Zellweger's disease. Infants with extremely low levels of DHA in the brain caused by Zellweger's disease, show progressively severe neurological dysfunctions. In addition, and even more convincing, supplementation of DHA markedly improves the symptoms of these children[56].

1.2.2 Subclinical deficiency

Current Western diet is possibly DHA-deficient as compared with our ancient diet

An interesting evolutionary view concerning the importance of LCPUFA availability for brain development of our ancestors has emerged. According to this view, humans with large brains could not have been evolved without the availability of abundant sources of LCPUFAs during the evolution of the human brain[57]. Archaeological evidence thus far supports an African origin of modern humans that settled near coastlines where plentiful LCPUFA rich food was available (fish, shellfish and marine sea bird eggs)[58]. Terrestrial food resources may not supply sufficient amounts of LCPUFAs to maintain a brain which has evolved from 450 g to 1.3 kg[57]. Others criticize the premise that an aquatic diet was necessary to maintain the evolvement of the human brain[59]. According to Langdon various premises behind the so called *n*-3 hypothesis are unsupported. Firstly, variations in DHA availability in human nervous tissue do only induce subtle and generally reversible effects on brain function. Secondly, the aquatic food chain is not the only effective dietary source for DHA. Estimates of the composition of hunter-gatherer and ancestral diets show that the diets were more nutritional balanced and healthy compared with the current Western diet. Thirdly, prolonged dietary deficiency of DHA is buffered by various compensatory mechanisms such as shifting DHA in and out of other body tissues and the (very low) endogenous synthesis of DHA from ALA. Fourthly, DHA availability is not believed to be the rate-limiting step in animal and human brain evolution. For instance, the elephant has

the largest terrestrial brain (with large amounts of DHA) and subsists on a great variety of terrestrial plant foods containing trace amounts of DHA[59]. Although the brain/bodyweight ratio in men is much higher than in the elephant, the absolute quantities of DHA accumulated in the brain are much higher in the elephant than in men. Since the agricultural revolution some 10,000 years ago, we gradually changed our diet. From the beginning of the industrial revolution 100-200 years ago, our diet has changed even more compared with our ancestral diet. Among numerous other changes in diet composition, we consume nowadays considerable less *n*-3 polyunsaturated acids[60]. For instance, the dependence on agricultural grains and grain-fed animals induced a higher *n*-6/*n*-3 ratio in our diet[59]. The considerable changes in diet composition and lifestyle since the industrial revolution may explain numerous 'typically Western diseases', such as cardiovascular disease, diabetes mellitus type 2, osteoporosis and certain types of cancer[14]. From an evolutionary point of view, the relatively fast changes in our diet and environment since the agricultural revolution could not have resulted in adjustments of our genome to our contemporary environment. An intriguing hypothesis is that our genome has not yet adapted to the low *n*-3 LCPUFA intake, or a high ratio of *n*-6/*n*-3 LCPUFA intake, which could have – amongst others - unfavourable consequences for brain development.

Early nutrition and subclinical LCPUFA deficiency

Although linoleic acid and α-linolenic acid deficiency is extremely rare under normal physiological circumstances, the relatively inefficient conversion of parent essential fatty acids into LCPUFAs and the relatively low intake of *n*-3 polyunsaturated fatty acids could lead to a state of subclinical deficiency, which cannot be detected at the individual level. It has been surmised that the low *n*-3 polyunsaturated fatty acid status could explain the increase in typical Western chronic diseases like cardiovascular and psychiatric diseases (e.g. ADHD and mood disorders)[14]. The focus of this thesis is whether LCPUFAs, especially *n*-3 LCPUFAs could be conditionally essential for normal brain function during the brain growth spurt[40]. Formula fed infants who do not receive formula feeding with LCPUFAs have a lower LCPUFA content in the cerebral cortex than infants who received breastfeeding, as has been studied in children who have died from the sudden infant death syndrome[19,46]. Furthermore, our group has demonstrated that a lower prenatal LCPUFA status negatively affects the neurological condition immediately after birth[62]. Infants with an abnormal neurological condition (n=27) had a higher DHA deficiency index and lower essential fatty acid index in their umbilical veins than neurologically normal infants. Furthermore, AA, the DHA suficiency index and the essential fatty acid index were positively associated with the neurological optimality score. The latter consists of the summation of all scores considered to be optimal (max 60 items) of the neurological examination according to Prechtl[63]. Whether a lower LCPUFA status has an influence on neurodevelopmental outcome at later ages and whether LCPUFA supplementation leads to a better neurodevelopmental outcome after birth is discussed in the next section.

1.3 Overview of evidence of the effect of LCPUFA supplementation on neurodevelopment

1.3.1 Evidence from animal experiments

A recent review of McCann & Ames in 2005, summarizing all evidence from cognitive and behavioural tests (visual function studies were excluded) in animals and humans, concluded that animal studies provided the most convincing and consistent evidence that DHA deficiency during development impairs cognitive and behavioural performance in early life. The authors also noted that the observed effects under severe dietary conditions were not substantially large and that these results are difficult to extrapolate to the human situation[50]. Another review concluded that animal studies have shown that the brain retains both DHA and AA even during prolonged dietary deficiency[64]. When DHA concentrations in brain tissue drop, it is accompanied by an increase in *n*-6 polyunsaturated fatty acids that maintains the total polyunsaturated fatty acids in the brain constant. However, during extreme *n*-3 fatty acid depletion resulting in reduced DHA concentrations in nervous tissue, cognitive and behavioural changes in animals has been observed[64]. These animal studies point at a possible functional role of DHA in the nervous system. This overview is focused on the effects of LCPUFA supplementation during early human development. For more details about animal studies on LCPUFA deficiency, the reader is referred to the review of Innis and McCann & Ames[50,64]

Animal supplementation studies that evaluate developmental outcomes using dietary circumstances resembling the human situation are rare (Table 2). One rodent study could not demonstrate an effect of DHA and/or AA supplementation during postnatal day 5-18 compared to a standard diet on water maze performance and working memory at 6 and 9 weeks, although supplementation resulted in higher LCPUFA content in the brain (Table 2)[65]. Another rodent study evaluated the effect of a diet containing essential fatty acids enriched with fish oil (*n*-3 LCPUFA rich) or palm oil (saturated fatty acid rich) during the pre- and postnatal period on various behavioural outcomes at different ages (young/adolescent mice, middle aged mice and old mice) (Table 2). They confirmed that there was no effect on the performance in the water maze test, but they did find that the fish oil enriched diet induced more explorative and more active avoidance behaviour in young/adolescent mice on the first day of the diet compared with the palm oil diet[66]. However, less explorative and active avoidance behaviour was found in the fish oil supplemented group at old age (17-19 months). To the best of our knowledge, only one study evaluated the effect of LCPUFA supplementation on motor development in primates. One of the reasons why the authors evaluated LCPUFA supplementation in non-human primates and not in human subjects was to reduce variability as much as possible in the study population. Furthermore, they studied the effects of higher amounts of LCPUFAs (0.9 wt% DHA and 1.0 wt% AA) than any prior human infant study. Previous studies used concentrations that are considered to be more ecological valid (see also section 1.1.5). The rhesus macaque infants who received LCPUFA supplementation for 6 months exhibited stronger orienting and motor skills than infants who received standard formula at postnatal day 7 and 14. No effect was found on activity or state-control[67].

TABLE 2. Overview of animal LCPUFA supplementation studies which resemble realistic dietary human circumstances (1999-2004).

Authors	Subjects	Groups[1]	DHA content (wt%)	AA content	Duration of supplementation	Testing age	Assessments	Results[2]
Wainwright et al 1999[65]	Long-Evans rat pups	BF E_1 E_2 SF*	- 2.5 -	2.5 - -	Postnatal day 5-18	6 and 9 wk	Morris water-maze Working memory test	BF=E_1=E_2=SF SF<BF E_1=E_2=BF=SF
Carrié et al 2000[66]	Swiss OF-1 mice	SF (n=66)† E (n=75)	0.94§	0.11	Whole prenatal period + till testing ages	Young (7-11 wk) Mature (9-11 m) Old (17-19 m)	Growth and development Open field Habituation Morris water maze Active avoidance	Weight: E>SF Young: E>SF Level of activity: Young; E>SF Mature; E=SF Old; E<SF, E=SF Young; E>SF
Champoux et al 2002[67]	Rhesus macaques infants	C (n=13) E (n=14)	- 0.9	0.04 1.0	Until 6 months of age	Postnatal day 7, 14, 21, and 30	Adjusted Brazelton Neonatal Assessment Scale	Day 7 and 14; motor maturity and orientation: E>C

[1]BF = breastfed group, E = experimental formula with LCPUFA, SF = saturated fatty acid group, C = control formula with ALA and LA.
[2]E<C: experimental formula significantly better outcome than control formula; E=C: no significant difference between experimental and control formula group.
* Number ranged from 19-21 per group
† Number of subjects at follow-up at 18 m (+/- 50% mortality)
§ Fish oil

1.3.2 LCPUFAs and retinal function

Almost 36% of the fatty acids of the retina is composed of LCPUFAs. DHA is especially highly concentrated in retinal tissue (20%), and even more in the retinal photoreceptor outer-segments (50%)[68,40]. When light hits the photoreceptor rhodopsine-retinal complex, which is embedded in the DHA rich membrane of the outer rod photoreceptor, a quick change in the structure of the complex occurs which involves membrane conformational changes. It is assumed that the function of the high DHA content of the retinal photoreceptor outer-segments is to facilitate the high frequency of conformational changes in the membrane surrounding the visual pigment rhodopsin[69]. Dietary changes in DHA intake only modestly induce modifications in the DHA content of the retina[40]. In DHA-deficient animals no alterations in the rate of light-induced rhodopsin conformational changes was observed, but DHA-deficiency impaired the photon-catching ability of rhodopsine[40]. The exact functional properties of DHA in the retina are far from clear.
The effects of LCPUFA, notably DHA on retinal function have been extensively studied in animals and human infants; because visual function is one of the easiest ways to objectively assess the function of the central nervous system. *N*-3 fatty acid depletion studies in animals have indicated that *n*-3 fatty acids are important for the development of optimal visual function. Some studies indicated that *n*-3 fatty acid depletion leads to irreversible impairment of visual function during early development[40]. Lauritzen et al. 2001 concluded that it is unwise to extrapolate the results of animal studies with extreme depletion of *n*-3 fatty acids to non-clinical human infant conditions. To our knowledge two new studies have been published since 2001 regarding the effect of LCPUFA supplementation on visual function of infant monkeys which resemble human LCPUFA supplementation studies

(Table 3). The study of Jeffrey et al. did not find a beneficial effect of postnatal LCPUFA supplementation on visual evoked potentials (VEP) or on the electroretinogram of term rhesus monkeys at 3 months[70,71]. The study of Diau et al. found that preterm infant baboons which did not receive LCPUFA supplemented formula feeding had worse electroretinograms than preterm infant baboons receiving LCPUFA supplemented formula feeding until 4 wk after term age (Table 3)[72]. Furthermore, the DHA levels in the retina of the baboons in this study were positively correlated with the retinal response.

TABLE 3. Two recent LCPUFA supplementation studies of infant monkey studies resembling human dietary conditions.

Authors	Subjects	Groups[1]	DHA content (wt%)	AA content (wt%)	Duration of supplementation	Testing age	Assessments[2]	Results[3]
Jeffrey et al. 2002[70]	Infant rhesus monkeys	C (n=?) E (n=?)	1.0	1.0	3 m	3 m	VEP ERG (23 parameters) Plasma DHA and AA	C=E C=E Both DHA and AA increased
Diau et al. 2003[72]	Preterm and term infant baboons	BF (n=4) E (n=4) P (n=4) PE (n=4)	0.68 0.61	0.62 1.21	Until the age of 4 wk after term age	0 and 4 wk adjusted age	ERG (0wk) ERG (4wk) Retinal fatty acid concentrations	No differences BF>C, BF, C, PE > P DHA: 19% less in C and P compared with B B=PE No effect of prematurity: P=C AA: BF=C=P=PE

[1]BF = breastfed group, E = experimental formula with LCPUFA, C = control formula, P = preterm/formula-fed, PE = preterm with experimental formula with LCPUFA.
[2]VEP = visual evoked potentials, ERG = electroretinogram.
[3]E<C: experimental formula significantly better outcome than control formula; E=C: no significant difference between experimental and control formula group.

In summary, the large amount of evidence from animal studies, together with support from basic research of the biophysical properties and biological functions of LCPUFAs lead to the conclusion that LCPUFAs are needed for an optimal functioning of the central nervous system. Total restriction of *n-3* fatty acids in early life results in behavioural and visual dysfunctions in animals[73]. Nevertheless variations in LCPUFA status might not significantly affect neurodevelopment in physiologically relevant human circumstances, possibly because of the presence of compensatory mechanisms such as an increase in 22:5n-6 brain content. To test the hypothesis that LCPUFA supplementation has a beneficial effect on neurodevelopment in circumstances relevant to humans, large randomized controlled trials are needed, which are discussed in the next section.

1.3.3 Overview of randomized clinical trials regarding LCPUFA supplementation on visual and neurodevelopmental outcomes

1.3.3.1 Overview of supplementation studies during pregancy

A few intervention studies evaluate the effects of LCPUFA supplementation during pregnancy and later developmental outcome of the child. To summarize, Helland et al.[74] found that cod liver oil supplementation (rich in *n*-3 fatty acids, 2494 mg/day; n=41) during pregnancy and lactation resulted in higher concentrations of *n*-3 polyunsaturated fatty acids at birth, at 4 weeks and 3 months of age compared with a corn oil supplementation group (rich in *n*-6 fatty acids; n=35). At the age of four years, children born to mothers receiving cod liver oil scored higher on the Mental Processing Composite of the Kaufman Assessment Battery for Children than children born to mothers receiving corn liver oil[74]. Note that the maternal supplementation was also given during lactation until 3 months after delivery[74]. The maternal DHA supplementation study of Malcolm et al.[75] during pregnancy (n=100) indicated that DHA supplementation (200 mg/day) did increase the maternal DHA status but not the DHA status of the infant at birth. They found an effect of maternal DHA supplementation on visual evoked potential maturation in healthy term infants compared with infants who were born to mothers who received a placebo capsule. In addition, they found an association between the DHA status of infants at term and early postnatal development of the pattern-reversal visual evoked potentials[75]. According to these findings, the DHA status of the infant itself could better explain the observed improved visual maturation of the infants than maternal DHA status which was either enhanced with DHA supplementation or not.

Two non-supplementation studies indicated that prenatal DHA status is not related to cognitive outcome: umbilical venous plasma and red blood cell phospholipid DHA and AA concentrations at birth were not related to cognitive outcome at 4 and 7 years of age[76,77].

A study of De Groot et al.[78] investigated the effect of α-linolenic acid (precursor of DHA) supplementation during pregnancy on the woman's cognitive functioning. No evident positive effect of maternal α-linolenic acid supplementation was found on the maternal DHA status[79]. In addition, no effect was found on cognitive outcomes at various ages until 32 weeks after delivery. De Groot et al. even found an unexpected adverse association between plasma DHA levels at 14 weeks of pregnancy and at 32 weeks after delivery with reaction times on the finger-precuing task[78].

The above data indicate that we still know relatively little about the relationship between prenatal LCPUFA status and neurodevelopmental outcome. In order to test the hypothesis that a higher prenatal LCPUFA status enhances neurodevelopmental outcome, we measured the fatty acid composition umbilical walls immediately after birth. The fatty acid composition of the umbilical vessels enabled us to relate prenatal fatty acid status with neurodevelopment at 3 and 18 months. These relationships are discussed in chapter 3.

1.3.3.2 Human LCPUFA supplementation studies and visual function

Results of human postnatal LCPUFA supplementation studies are in line with the results obtained from animal studies. The review of Lauritzen et al.[40] indicated that all four randomized trials in preterm infants on LCPUFA supplementation showed improved visual function in the supplemented group. The results of studies evaluating LCPUFA supplementation on visual function of term infants show inconsistent results[40,80]. An extensive review of San Giovanni based on 14 studies showed that in term infants the

positive effects are only relevant at two months and negligible thereafter[80]. However a recent large study of Birch et al.[81] demonstrated that LCPUFA supplementation (0.36% DHA and 0.72% AA) till the age of 52 weeks induced better visual function as measured by visual evoked potentials (VEPs) at the ages 6, 17, 26, and 52 weeks of healthy term infants. Study characteristics which might explain the positive outcome of this study are the long duration of supplementation, the relatively high DHA concentration in the experimental formula group, the relatively large sample size (n=103), and use of a relatively sensitive outcome parameter (VEP)[81]. To date, no comparable studies are known which can confirm these remarkable findings. To summarize, LCPUFA supplementation improves visual development in preterm infants and possibly in term infants.

1.3.3.3 Human LCPUFA supplementation studies and neurodevelopmental outcomes

Numerous studies haven been performed to evaluate the effect of LCPUFA-supplementation on neurodevelopment. Compared with the evidence regarding the effects of LCPUFA supplementation on visual function, the effects on neurodevelopment are less pronounced. LCPUFA supplementation studies in preterm infants show inconsistent results. Table 4 shows the study characteristics of all randomized trials regarding LCPUFA supplementation in preterm infants. The studies are rank-ordered according to DHA-content of the supplementation. Two Cochrane meta-analyses (updated until October 2003) concluded that that there is insufficient evidence to show that supplementation improves visual function, neurodevelopment and cognition of preterm and term infants[82,83]. Since then, two additional randomized controlled trials investigating LCPUFA supplementation in preterm infants have been published. Clandinin et al.[8] found a beneficial effect of LCPUFA supplementation for 52 weeks on neurodevelopment at 18 months of age. However the study of Fewtrell et al.[85] with a similar design could only find a beneficial effect on the mental developmental index (MDI) of boys, but not in the total study population[85]. The longer duration of supplementation in the study of Clandinin et al.[84] compared with the study of Fewtrell et al.[85] could explain the inconsistent results of the studies. In addition, the high attrition rate of both studies precludes firm conclusions.

Only a minority of LCPUFA supplementation studies in term infants showed positive results at early age. A review of Hadders-Algra in 2005 indicated that postnatal LCPUFA supplementation in term infants may exert beneficial effects on neurodevelopmental outcome till 4 months of age, but not beyond (See table 4 and 5 for an updated version of all LCPUFA supplementation studies till 2006 which are rank-ordered according to the DHA-content of the supplementation)[86]. Studies, which used relatively high DHA concentrations ($\geq$ 0.30%), showed more positive results of LCPUFA supplementation than studies using LCPUFA in lower concentrations. Two high quality Cochrane meta-analyses investigating the effects of LCPUFA supplementation in preterm (updated until October 2003) and term infants (updated until June 2001) conclude that there is insufficient evidence for a beneficial effect of LCPUFA supplementation on neurodevelopment[82,83]. No definite conclusion can be made on the effect of LCPUFA supplementation on various neurodevelopmental outcomes, because of inconsistent results that in turn could be explained by specific study characteristics such as dosage, duration of supplementation, type of assessment and age at investigation. Nevertheless, the general picture emerges that LCPUFA supplementation induces subtle differences in developmental outcome at most. This conclusion does not rule out that such subtle effects could have a relevant impact in the general population. At present, no studies have addressed the long-term effects of LCPUFA supplementation at school age.

1.3.4 Concluding remarks

LCPUFAs are important constituents of the central nervous system. The high accumulation of LCPUFAs in brain tissue during prenatal and postnatal development together with the fact that breastfeeding contains relatively high amounts of LCPUFAs led to the hypothesis that LCPUFA supplementation in standard formula feeding might be beneficial for infant neurodevelopment. Many double blind randomized controlled trials have been performed to evaluate the potential positive effects of LCPUFA supplementation in both preterm and term infants. Until now, a relevant beneficial effect of LCPUFA supplementation of preterm infants on visual development has been found in several high quality randomized trials. Whether LCPUFA supplementation during early life also exerts effects on other neurodevelopmental outcomes is still debated. It seems that LCPUFA supplementation under normal Western human circumstances has only subtle transient beneficial effects on neurodevelopment at early ages; a conclusion that is consistent with the results of the double blind randomized controlled trial subsequentely described in the present thesis.

TABLE 4. Overview of LCPUFA supplementation studies in preterm infants.

Author	Participants	Groups	Duration of supple-mentation	DHA content %wt	AA content %wt	Age at FU in mo	Attrition at last FU	Assessment at FU	Results
Hansen et al.[87] 1997‡	BW 0.86-1.56kg	E1, E2, C } Randomized: n=194 BF n=80	?	0.15% 0.14%	0.27%	2 and 4 mo PCA	?	Teller	E1, E2=C (BF?)
Fewtrell et al.[88] 2002	BW <1750g GA 30 ±2wk	E n=95 C n=100 BF n=88	33 ± 17 days	0.17%	0.31%	9, 18 mo post term	15%	BSID KDSI Neurological impairment	E=C=BF BF>E, C E=C=BF
Carlson et al.[89] 1992	GA 29 ±2wk	E n=33 C n=34	From birth till 9 mo of age	0.2%	-	Term age till 12 mo after term	15%	VEP Fagan	E=C At 9 mo: total looks E>C At 12 mo: novelty time E<C; total looks E>C
Carlson et al.[90] 1996	BW between 747-1275g Subgroup had BPD (40%)	E, C } Total: n= 94	3-5 d of age to 48 wk PCA	0.2%	-	0, 2, 4, 6, 9, and 12 mo after term	37% at 4 mo later: ?	Teller Fagan	At 2 mo: E<C E=C
Faldella et al.[91] 1996*	AGA, >50% enteral feeding on day 10 GA 31 ±1wk	E n=23 C n=26 BF n=17	Until 52 wk PCA	0.23%	0.35%	52 wk PCA	12%	VEP§ BAEP	At 52 wk PCA: E, BF>C; BF=E* E=C=BF
O'Conner et al.[92] 2001‡	GA < 33wk and between 750-1805g	E1 n=140 E2 n=143 C n=144 BF n=43	Till 12 mo after term	0.26% 0.26%	0.42% 0.42%	2, 4, 6, 9, 12, 14 mo corrected age		BSID Teller VEP Fagan MCDI	E1, E2=C=BF E1, E2=C=BF E1, E2=C=BF E1, E2=C=BF E1, E2=C=BF
Clandinin et al.[93] 2005		E1 n=112 E2 n=130 C n=119 BF‖ n=105	Till 52 wk after term	0.3% (algal) 0.3% (fish)	0.64-0.67% 0.64-0.67%	18 mo after term	45%	BSID	E1, E2 > C BF > E1, E2, C
Van Wezel et al.[94] 2002	GA 30 ±2wk	E n=22 C n=20	Until 6 mo corrected age	0.34%	0.68%	3, 6, 12, 24 mo corrected age	0%	Cerebral myelination BSID VEP Teller	E=C E=C E=C E=C
Innis et al.[95] 2002‡	BW 846-1560g Healthy preterm infants GA 30 ±2wk	E1 n=66 E2 n=66 C n=62	>28 days till discharge	0.34% 0.33%	- 0.60%	40, 48 and 57 PCA	≈25%	Teller	E1, E2=C
Uauy et al.[96] 1990	GA 31 ±2wk	E n=12 C1 n=10 C2 n=10 BF n=10	From day 10 to 36 wk PCA	1.0% (0.65% EPA) 0 (corn oil) 0 (soy oil)	0 0 0.1%	36 and 57 wk PCA	5%	ERG Bleeding times	36 wk: C1, C2>E E=C1, C2
Fewtrell et al.[97] 2004		E n=122 C n=116	Till 9 mo after term	0.5% (tuna fish oil)	0.04%	9, 18 mo after term	49%	BSID KDSI	E=C; Boys MDI: E > C E=C

E = experimental formula with LCPUFA, C = control formula without LCPUFA, BF = breastfed group. (A)GA = (adequate) gestational age, mean ± SD; PCA = post conceptional age; EPA = eicosapentaenoic acid; ERG = electroretinogram; VEP = visual evoked potential; Fagan = Fagan Test of Intelligence; BW = birth weight; BPD = bronchopulmonary dysplasia; Teller = Teller Acuity Card procedure; BAEP = Brainstem auditory evoked potentials; BSID = Bayley Scales of Infant Development; MCDI = McArthur Communicative Development Inventories; KDSI = Knoblock, Passamanik & Sherrard's Developmental Screening Inventory.
*Randomization and intervention were not blinded. ‡Unclear whether assessment was blinded. §Methodology of assessment for VEP and ERG deviate from international standards ‖Term infants' reference breastfed group.

TABLE 5. LCPUFA supplementation in term infants and outcome until 4 months[86]

Author(s)	Groups	Duration of supplementation	DHA content	AA content	Age at FU in mo	Attrition at last FU	Assessment at FU	Results
Carlson et al.[98] 1997	E n=19 C n=20 BF n=19	? BF ≥ 3 mo	0.10%	0.43%	2 and 4	38%	Teller visual acuity	2 mo: E>C; BF > C 4 mo: E=C; BF = C
Auestad et al.[99] 2001	E1 n=58 E2 n=60 C n=56 BF n=120	12 mo BF ≥ 3 mo	0.14% (egg) 0.13% (fish/fungal)	0.45% 0.46%	1,2 and 4	27% (at 12 mo)	Teller visual acuity	E1, E2=C, BF=C
Auestad et al.[100] 1997	E1 n=26 E2 n=28 C n=28 BF n=38	≥ 4 mo BF ≥ 4 mo	0.12% 0.20%	0.43%	2 and 4	39% (at 12 mo)	FPL Sweep VEP	E1, E2=C; BF=C
Agostoni et al.[101] 1995	E n=27 C n=29 BF n=30	4 mo BF ≥ 4 mo	0.30%	0.44%	4	4%	Brunet-Lezine Development Quotient	E>C BF>C
Bouwstra et al.[102] 2003	E n=131 C n=119 BF n=147	2 mo BF variable; median 9 wk	0.30%	0.45%	3	16%	Quality of general movements	E>C BF>C
Makrides et al.[103] 2000	E1 n=24 E2 n=23 C n=21 BF n=46	12 mo BF ≥ 3 mo	0.34% 0.35%	0.34%	4	18% (at 8 mo)	VEP	E1, E2=C; BF=C
Makrides et al.[104] 1995	E n=13 C n=19 BF n=23	? BF ≥ 4 mo	0.36%		4	11%	VEP	E > C BF>C
Birch et al.[105] 1998	E1 n= 23 E2 n=22 C n=23 BF n=21	4 mo BF ≥ 4 mo	0.36% 0.35%	0.72%	1.5 and 4	18%	FPL Sweep VEP	E1, E2=C; BF=C E1, E2>C; BF>C
Birch et al.[81] 2005	C n=52 E n=51	12 mo	0.36%	0.72%	6,17,26,52 wk	17%	Sweep VEP	E > C
Ünay et al.[106] 2004	E n=22 C n=22 BF n=23	4 mo BF ≥ 4 mo	0.50%		4	16%	BEAP	E > C BF>C
Jensen et al.[107] 2005	BC n=113 BE n=114	4 mo Maternal supplementation during lactation	≈200 mg DHA/d	-	4	22% (at 4 mo)	Teller visual acuity VEP	BC=BE BC=BE (latency) BC > BE (amplitude)

E = experimental formula with LCPUFA, C = control formula LCPUFA, BF = breastfed group, BC = Breast feeding control group, BE = Experimental maternal supplementation breastfed group. FPL = Forced-choice preferential-looking test; VEP = visual evoked potential; BAEP = Brainstem auditory evoked potentials.

TABLE 6. LCPUFA supplementation in term infants and outcome beyond 4 months[86]

Author(s)	Groups	Duration of supplementation	DHA content	AA content	Age at FU in mo	Attrition at last FU	Assessment at FU	Results
Carlson et al.[108] 1996	E n=19 C n=20 BF n=19	? BF ≥ 3 mo	0.10%	0.43%	6 and 12	> 38%	Teller visual acuity	E=C BF=C
Auestad et al.[99] 2001	E1 n=58 E2 n=60 C n=56 BF n=120	12 mo BF ≥ 3 mo	0.14% (egg) 0.13% (fish/fungal)	0.45% 0.46%	6, 9, 12	27% (at 12 mo)	Teller visual acuity Fagan Bayley PDI/MDI IBQ Language	E=C; BF=C E=C; BF=C E=C; BF=C E=C; BF=C E=C; BF=C
Auestad et al.[100] 1997	E1 n=26 E2 n=28 C n=28 BF n=38	≥ 4 mo BF ≥ 4 mo	0.12% 0.20%	0.43%	6, 9, 12	39%	FPL Sweep VEP	E=C BF=C
Scott et al.[109] 1998	E1 n=38 E2 n=33 C n=42 BF n=60	≥ 4 mo BF ≥ 3 mo	0.12% 0.20%	0.43%	12 14	37%	Bayley PDI/MDI McArthur language	E1=C; E2=C; BF=C E1=C; E2 < C; BF=C
Willatts et al.[110] 1998	E n=21 C n=23	4 mo	0.20%	0.30%	10	38%	Problem solving task	E >
Auestad et al.[111] 2003	E1 n=35 E2 n=35 C n=37 BF n=50	12 mo BF ≥ 3 mo	0.12% 0.23%	0.43%	39	47%	Teller visual acuity Beery VMI Stanford-Binet IQ Language	E=C; BF=C E=C; BF=C E=C; BF=C E=C; BF=C
Bouwstra et al.[112] 2005	E n=135 C n=157 BF n=154	2 mo BF variable; median 9 wk	0.30%	0.45%	18	6%	Hempel neurological examination Bayley PDI/MDI	E=C; BF=C E=C; BF=C
Agostoni et al.[113] 1997	E n=26 C n=30 BF n=25	4 mo BF ≥ 4 mo	0.30%	0.44%	24	10%	Brunet – Lezine DQ	E=C;BF=C
Lucas et al.[114] 1999	E n=125 C n=125 BF n=104	6 mo BF ≥ 6 wk	0.32%	0.30%	9 18	21%	Knobloch DQ Bayley PDI/MDI	E=C; BF=C E=C; BF=C
Makrides et al.[103] 2000	E1 n=19 E2 22 C 19 BF 23	12 mo BF ≥ 3 mo	0.34% 0.35%	0.34%	8, 12 and 24	12%	VEP Bayley PDI/MDI	E=C; BF=C PDI: E=C; BF=C 12 mo MDI: E=C; BF=C; 24 mo MDI: E=C; BF > C
Birch et al.[105] 1998	E1 n=19 E2 n=22 C n=20 BF n=46	4 mo BF ≥ 4 mo	0.36% 0.35%	0.72%	6 and 12	26%	FPL Sweep VEP	E=C; BF=C 6 mo VEP: E=C; BF=C; 12 mo VEP: E1>C; E2>C; BF>C
Birch et al.[115] 2000	E1 n=17 E2 n=19 C n=20	4 mo	0.36% 0.35%	0.72%	18	28%	Bayley PDI/MDI	PDI: E1=E2=C MDI: E1 > C; E2=C
Birch et al.[81] 2005	C n=52 E n=51	12 mo	0.36%	0.72%	6, 17, 26, 52 wk	17%	Sweep VEP Random dot stereoacuity	6,17,26,52 wk: E > C 17 wk: E > C
Jensen et al.[107] 2005	BC n=113 BE n=114	4 mo Maternal supplementation during lactation	≈200 mg DHA/d	-	4, 8, 12, 18	30%	Teller visual acuity VEP Gesell Gross Motor DQ CAT DQ CLAMS DQ Bayley MDI/PDI	4 and 18 mo BC=BE BC=BE (latency) BC > BE (amplitude) 12 and 30 mo BC=BE 12 and 30 mo BC=BE 12 and 30 mo BC=BE 30 mo MDI: BC=BE 30 mo PDI: BE > BC
Lauritzen et al.[116] 2005	BC* n=60 BE n=62 BR n=53	4 mo Maternal supplementation during lactation	≈900 mg DHA/d	-	9, 12, 24	37%	Problem solving ability (IPT) MacArthur Communicative Development Inventory	9 mo: BC=BE=BR 12 mo vocabulary comprehension: BE < BC, BR=BC, BE 24 mo: BC=BE=BR Boys sentence complexity: BE < BC

IPT = Infant Planning Test
BC = Breast feeding control group
BE = Experimental maternal supplementation breastfed group
BR = Reference breastfed group whose mothers have a high fish intake (upper quartile)
*Breast feeding control group whose mothers have a low fish intake (below population median)

1.3.5 Infant requirements of essential fatty acids

To estimate the requirements of essential fatty acids for infants, the US Food and Nutrition Board (Institute of Medicine of the National Academies) uses several indicators[117]. The Recommended Dietary Allowance (RDA) is used to indicate the average daily dietary nutrient intake sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group. Because no sufficient scientific evidence is available to conclude on a RDA for *n*-6 and *n*-3 polyunsaturated fatty acids, the Adequate Intake (AI) is used, instead of the RDA. The AI indicates the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake for a group of apparently healthy people that are assumed to be adequate. In addition to the recommendations of the Food and Nutrition Board, a workshop was held to establish the recommended dietary intakes for *n*-6 and *n*-3 fatty acids that also included specific AIs for DHA, eicosapentaenoic acid and AA[118]. These AI values are also included in table 6. Note that a potential drawback of the AI indicator is that systematic historical shifts in the mean intake of specific nutrients that could have general negative effects on health are not taken into account. Observational, epidemiological and experimental studies are needed to demonstrate lower and upper limits of dietary intake of essential fatty acids that could harm infant health, growth or development.

TABLE 6. Adequate Intake (AI) for infants aged 0-12 months

Adequate intakes of the US Food and Nutrition Board[117]

Fatty acid	Life stage group	Criterion	AI (g/d)
Total *n*-6 PUFAs	0 through 6 months	Average consumption of total *n*-6 fatty acids from human milk	4.4
	7 through 12 months	Average consumption of total *n*-6 fatty acids from human milk and complementary foods	4.6
Total *n-3* PUFAs	0 through 6 months	Average consumption of total *n*-3 fatty acids from human milk	0.5
	7 through 12 months	Average consumption of total n-3 fatty acids from human milk and complementary foods	0.5

Workshop on the essentiality of and recommended dietary intakes for *n*-6 and *n*-3 polyunsaturated fatty acids[118]

Fatty acid	Percentage of fatty acids of infant formula or diet
Linoleic acid	10.00
α-linolenic acid	1.50
AA	0.50
DHA	0.35
EPA (upper limit)	<0.10

1.4 Various ways to assess neurological development

1.4.1.1 Introduction

The assessment of neurological development generally has three main purposes; (1) to diagnose neurological disorders with or without significant developmental delay and (2) to evaluate the changes in neurological condition over time and (3) to compare outcomes with typically developing healthy children. Various instruments can be utilized to assess neurological development in clinical and research situations. For instance, the neurological examination according to Touwen can both be used as an instrument to detect neurological disorders and as an instrument to monitor neurological condition over time in children aged 4 to 18 years[119]. Furthermore, the Van Wiechen assessment, which is widely used in The Netherlands, is an example of a neurological instrument, which is suitable for the screening of major developmental delays, but is not regarded appropriate for evaluating subtle differences in neurological condition[120]. The selection of the appropriate instrument is therefore dependent on the initial purpose of the neurological investigator. When applied to the present thesis, the neurodevelopmental assessments should be sufficiently discriminative, even under non-pathological circumstances in order to study the influences of nutrition on neurological development in a healthy term population of infants.

In general, the measurement of neurological development should be objective, which means that both reliability and the validity should be sufficient. Several issues emerge considering the measurement of neurological condition in developing children. Until now, no 'crucial' simple neurological test is available which can measure the overall integrity of the brain[119], because the central nervous system is a complex system consisting of many intertwined subsystems. Only a few subsystems can be tested independently. In general, the significance and predictive values for observational neurodevelopmental assessments at early age are limited. This is not only due to minor random or systematic errors in the measurements, but also due to inherent physiological variability of the observational phenomena and dynamical developmental changes of the central nervous system over time. Therefore, the neurological assessment must be well standardized, e.g. the test conditions, the behavioural state of the child, and the scoring procedure must be clearly described to prevent unwanted interrater variability. The inherent neurophysiologic variability in the observations that often occur under normal circumstances during development and the occurrence of dynamical developmental changes in time must be accepted when measuring the neurological development of children. Those sources of uncertainty partly explain the relative unpredictability of neurological development. It is important to realize that until now, the professional literature concerning neurodevelopmental testing has paid relatively little attention to the inherent neurophysiologic variability in the responses as opposed to the well-studied random variability due to measurement errors and the changes in the neurodevelopmental outcomes at various ages. The aforementioned inherent limitations in assessing neurological development must be kept in mind when one reviews the psychometric properties of neurodevelopmental assessments that will be discussed in section 1.4.2.

The following considerations concerning the different approaches of assessing neurological development used in the present thesis are being discussed, namely; (1) the classical

approach, including the traditional neurological examination and the assessment of the onset of motor milestones and (2) the assessment of the quality of motor behaviour. The two approaches will be discussed below.

1.4.1.2 Approach I. Classical approach of assessing neurological development

The classical approaches to the assessment of neurological development are based on the ideas of the so-called neuronal-maturation theory developed in the mid-1900s. Important pioneers were Gesell and Amatruda who studied motor development of children. They believed that motor development could be regarded as the gradual unfolding of predetermined patterns in the central nervous system[121]. The maturation of brain regions were considered as the underlying mechanisms that caused the observed orderly onset of motor milestone achievements in developing children in a healthy general population. In addition, the maturation of cortical regions was considered responsible for the progressive inhibition of 'primitive reflexes' generated in the spinal cord and brain stem. Based on the premises that motor development was a fixed process that is determined by genetic processes in which environmental stimuli played a minor role, general developmental rules were formed, such as the cephalocaudal and central-to-distal sequences of development[122]. However, discoveries of critical or sensitive periods in neurodevelopment have indicated that at least a minimal amount of environmental stimuli was required for brain development of animals and humans. This convinced many investigators that environmental factors also played a role. For instance, during the 1960s, the Nobel Prize winners Hubel and Wiesel demonstrated the essential role of environmental visual stimulation in the formation of structure and function of the visual system[123,124]. These insights led to a general notion that a minimal amount of environmental input during a specific time window might be necessary for normal development of cortical regions. The acknowledgement that environmental input could improve motor development provided an important rationale for paediatric physiotherapists to develop intervention programs to facilitate motor behaviour of infants in an orderly prescribed way based on the developmental rules described by the neuronal-maturation theory. The developmental principles underlying the neuronal-maturation theory also provided the basis for the classic paediatric neurological examination and various methods to assess motor behaviour of children that provide important information concerning the integrity of nervous system. The classical neurological examination of children is generally composed of the following neurological domains; mental status, cranial nerves, the motor system (motor behaviour, active power, tone, reflexes, coordination, involuntary movements), and the sensory system[125,126]. Abnormal findings during the neurological examination usually localize the specific area(s) of neurological dysfunction and may confirm the working diagnosis. Many aspects of the classical neurological examination of children differ from that of adults, because of the age-specific expression of neuronal functions. The age-specific expressions in neuronal functions reflect the profound changes in the functional architecture of the developing brain that can be seen as the ongoing maturation of the brain. Therefore, interpretation of pathological neurological signs and symptoms must always be related to the developmental stage of the child. Neurological responses such as developmental reflexes or 'primitive' reflexes and postural reactions of infants are good examples of age-specific neurological expressions of the developing brain. The onset and achievement of motor, social and language developmental milestones are an integral part of the classical neurological examination of children[125,126]. Several instruments have been developed to assess developmental milestones, such as the Bayley Scales, Alberta Infant Motor Scale, Peabody

Developmental Motor Scales, Van Wiechen-assessment, and the Denver Developmental Screening Test[127-130]. All these instruments are norm-referenced measures to identify developmental delays. The instruments also are used as an evaluative tool used in research settings.
To summarize, the classical approach of assessing neurological development in children is based on the principles of the neuronal-maturation theory that states that neurological development is mainly determined by gradual maturation of innate structures in the central nervous system.

1.4.1.3 Approach II. Assessment of quality of motor development

The assessment of the quality of motor repertoires of children is a promising approach to assess neurological development of children with or without significant neurological abnormalities. Especially the observation of variation in motor behaviour is a sensitive instrument to assess the integrity of the central nervous system[131]. Normal development is characterized by abundant variation manifesting itself in variable motor repertoires, the duration of developmental stages and in the sequence of the achievement of developmental milestones[131]. Variation in motor repertoires manifests itself for instance when a 4-months-old child reaches for an interesting toy in many different ways. The absence of variability of motor repertoires and consequently the presence of stereotypic motor behaviour is associated with abnormalities in neurological development such as in cerebral palsy and minor neurological dysfunction[131,132]. Children with cerebral palsy, for example, show striking little variability in reaching and grasping during the development of those functions compared with typical developing children[133]. However, persistence of variability in reaching and grasping beyond the normal time window for the development of these functions might be observed in children with cerebral palsy.
In order to explain the occurrence of variation in motor development, Sporns and Edelman proposed a theoretical framework called the Neuronal Group Selection Theory (NGST)[135]. The NGST is based on the following assumptions: (1) variable functional groups of neuronal networks are organized in the central nervous system by genetic and epigenetic mechanisms that are formed by evolution. (2) Neuronal groups, which show self-generated variable activity, compete with each other for survival (primary variability). (3) The most efficient functional neuronal groups are being selected on the basis of innate selection criteria. (4) Variable activation patterns emerge from neuronal groups that are being activated by afferent neuronal information caused by task-specific circumstances (secondary variability). (5) Neuronal groups, which generate the most efficient motor solution, will be selected from a pool of neuronal groups and will be used again in similar task situations in the future, so that the best available motor solution for each specific motor task will be learned. The previous assumptions are supported by computer simulations of a simple motor system that works according the principle of selecting the most efficient motor solution out of variable motor repertoires described by Sporns and Edelman[135]. Note that this type of evidence is not sufficient for accepting that the principles of this artificial computer model will also be present in the human brain. Therefore, more evidence from natural neuronal networks are needed to support the NGST theory. Fundamental research in the field of neurodevelopmental biology has revealed that variability in properties of the functional neuronal networks are formed by dynamic epigenetic regulation of cell division, adhesion, migration, death and neurite extension and retraction[131]. These processes potentially could be influenced by nutritional influences such as LCPUFA supplementation. As already been described earlier in this thesis, incorporation of LCPUFAs in neuronal

membranes influence the general biophysical properties of the nervous system, especially in areas involved in motor tasks[33,72]. In addition, LCPUFAs modulating the regulation of gene expression of many genes involved in developmental processes in the brain[36]. It is known that during development, variable self-generated neuronal activity is present in the central nervous system[136]. Self-generated variable neuronal activity is caused by complex electrophysiological properties of ion-channels in neuronal tissues, which produces self-generated activity in neuronal networks[137]. The development of the mammalian visual pathway is a good model for illustrating that self-generated neuronal activity is necessary for synaptic refinement during early development[136]. Self-generated activity in the neuronal networks involved in the formation of visual pathways during early development has been documented in different animal species[138-143]. Evidence is accumulating that synapse formation on the basis of spatiotemporally correlated activity induces precise network wiring by selective survival of synchronous activity of synapses[144]. The exact mechanisms governing the selection of the best functional circuits remain to be elucidated but the selection could be governed by fixed genetic programs or by rewarding centres in the brain during the phase of secondary variability. All these fascinating findings support the NGST.

When the NGST theory is applied to observable motor phenomena of children, two phases of variability of motor activity can be observed; the primary and secondary phase of variability. The length of the primary phase and consequently the onset the secondary phase of motor variability may vary for each motor function. For instance, the primary variability phase of sucking motor behaviour is relatively shorter than that of reaching and grasping and as a result the onset of secondary variability in sucking behaviour is earlier than that of reaching and grasping behaviour. It is essential for survival that sucking behaviour, which is an important feeding motor skill, is fully functional after birth, whereas reaching and grasping behaviour is not necessary for survival immediately after birth. The primary phase of variability is characterized by means of self-generated neuronal activity, and consequently by self-generated afferent information which is not yet adapted to specific motor task situations as opposed to the secondary phase of variability which is adapted to specific motor tasks[131]. An illustrative example of primary variability in motor development is a specific class of spontaneous movements of the human foetus and young infant. This class of spontaneous movements is called general movements (GMs) which are characterized by endogenously generated motor patterns that occur without external stimuli. GMs consist of variable and complex movements occurring in all body parts[146-148]. The assessment of the quality of general movements can be used to measure neurological condition that will be discussed later on, especially when combined with other neurological tests[149-151]. After the phase of primary variability and selection of primary motor repertoires have occurred, motor variability manifests itself in another phase called secondary variability. During the phase of secondary variability, the child is increasingly able to select the most efficient motor pattern for a whole range of specific motor tasks. Ultimately, motor variability in goal directed behaviour is not prominent anymore, but can become manifest in learning new motor tasks[131,152].

The framework of the NGST is suitable to explain variability in normal motor behaviour of children in an age-specific manner. Knowledge regarding the developmental trajectory of the quality of variation in motor behaviour is available and could provide valuable additional knowledge, which can be used to distinguish normal from abnormal neurodevelopment in children. For a general overview of the developmental trajectories of the variability of motor functions, postural adjustment and language performance, see Touwen 1993 and Hadders-Algra 2000[131,132]. It should be kept in mind that the study of the

developmental course and expression of quality, including variability, of motor behaviour is still an ongoing field of research. Nevertheless, evidence is accumulating that there is an association between impaired quality of motor development and neurodevelopmental outcome. For instance, in a mixed group of infants with or without a high risk for neurodevelopmental problems, the observation of the variation, complexity and fluency of general movements (GMs) of infants until 3-4 months after term is a valuable instrument to assess the risk for the development of cerebral palsy or minor neurological dysfunction[149,150]. Furthermore, the assessment of the quality of neurodevelopment at later ages can be evaluated in a reliable and sensitive way by means of the methods described by Prechtl, Touwen, and Hempel examination[63,153,154]. A more detailed description of the neurological assessments used in the present thesis is presented in the next section.

1.4.2 Assessments of neurodevelopment in this thesis

Neurological assessment at 3 months: quality of General Movements

The evaluation of the quality of general movements (GMs) has been shown to be an promising technique for evaluating the quality of brain function in young infants[155-157]. GMs are complex movements that involve the head, trunk, arms, and legs which lack a distinctive sequencing of the body parts[157]. GMs arise during early foetal life and persist until 3–4 months after full term age. During prenatal and postnatal development the gestalt of GMs changes. Three distinct phases in the development of GMs can be described: (1) preterm GMs from about 28 to 36-38 weeks are characterized by extremely variable movements, including many pelvic and trunk movements; (2) writhing GMs from 36-38 to 46-52 weeks are characterized by somewhat forceful (writhing) movements that are superimposed on the variable movement patterns; (3) fidgety GMs from 46-52 to 54-58 weeks and can be described as a characteristic continuous flow of small and elegant movements occurring irregularly all over the body[157]. Normal GMs are characterized by fluency, variation, and complexity. These characteristics disappear when movements become abnormal. Movement fluency is the first property to disappear, which means that subtle dysfunctions of the nervous system already result in movements with a jerky or stiff appearance. Movement complexity and movement variation, which in fact can be considered as two forms of movement variation, are the major characteristics of GM quality. Four classes of GM quality can be distinguished: two forms of normal GMs (normal-optimal and normal-suboptimal) and two forms of abnormal GMs (mildly and definitely abnormal). The assessment of the quality of GMs consists of a standardized 15-minutes video recording of spontaneous mobility in the supine position. The inter-rater agreement on GM quality, determined with several random samples of videos was good (Kappa = 0.81)[150]. The quality of GMs during the last phase (fidgety phase; 2-4 months after term) is the best period to predict neurological condition at later age, as has been demonstrated by various prospective longitudinal studies in a mixed group of infants who have either a high or a low risk for neurodevelopmental disorders. These studies have indicated that infants who show definitely abnormal GMs during the last stage of GM-development (fidgety phase) have a high risk (70-95%) for the development of cerebral palsy[146,154,158,159]. One should be aware that the variable risks reported in the literature are possibly due to the slight differences in the criteria of definitely abnormal GMs used in the literature and the high a priori chance of the development of cerebral palsy and other developmental problems due to the mixed study population. Recent studies have demonstrated that infants with definitely abnormal GMs at fidgety age who do not develop cerebral palsy usually show other developmental problems, such as minor neurological dysfunction (MND) and behavioural problems[151]. Mildly abnormal GMs at 3 months of age are, to a limited extent, related to MND, attention problems and aggressive behaviour[149,150]. However, the positive predictive value is poor, because of the many false positive cases of children without neurological disturbances. The power to predict minor developmental problems improves considerably when combined with other neurological assessments such as neurological examination and the cranial ultrasound scan[151]. More longitudinal studies are needed in populations of low risk infants to assess the predictive value of the quality of GMs in infants without a high risk for developmental disorders. Future follow-up of the

healthy term infants in the cohort presented in this thesis give valuable insights into the significance of the quality of GMs in a representative low risk Dutch population.

1.4.2.1 Neurological assessments at 18 months

Bayley Scales of Infant Development
The Bayley Scales of Infant Development (BSID-II) is a discriminative instrument that assesses the mental and psychomotor development of children aged 1 month to 3.5 years[160]. The Bayley Scales are also used widely for evaluation purposes in the context of research[82,161]. The mental developmental index (MDI) and the psychomotor developmental index (PDI) score are based on the number of tasks successfully completed. The MDI assesses memory, problem solving, discrimination, classification, language, and social skills. The PDI measures control of gross and fine muscle groups, including walking, running, jumping, grasping, and imitation of hand movements. The scores are converted into age-normalized scores that can be used to compare children with known reference norms derived from several populations. The normalized scores presented in this thesis are derived from the recently developed Dutch norms[160]. An important limitation of the BSID-II motor scale is the inability to differentiate gross and fine motor development and the emphasis on cognitive motor performances rather than pure motor performance. As already discussed previously, the BSID-II is not designed to assess qualitative aspects of motor development. The inter-rater and test-retest reliability of the PDI and the MDI is good, although the reliability of the PDI is somewhat less than that of the MDI[162]. The concurrent validity of the BSID-II is good when age equivalent scores are used, but not when standardized scores are used[163-165]. The predictive validity of the BSID-II is poor which partly could be attributed to the inherent variance of the central nervous system itself which is analogous to a high biological variance (see also section 1.4.1.1)[166]. However, Hack et al. did demonstrate that the combination of low Bayley scores and severe abnormalities on cranial ultrasound are reliable early markers of poor cognitive outcome[166,167].

Examination according to Hempel
The Hempel assessment is a standardized assessment technique designed for the detection of minor signs of neurological dysfunction during toddler age (1.5-4 years)[154]. Hempel developed the examination in cooperation with professor Touwen during the early 1990s. The Hempel assessment does not only assess traditional signs of neurological dysfunction, such as mild abnormalities in muscle tone regulation and motor milestones, but also the quality of motor behaviour. Besides the ability to detect minor neurological dysfunctions, neurological abnormalities can also be detected. The examination regarding the quality of motor functions is based on criteria based items and age-normalized data. An integral part of the assessment is the observation of spontaneous motor behaviour in a free field play situation. This enables the investigator to observe the important qualitative aspects of spontaneous motor behaviour without compromising the cooperation of the child. The following motor functions are observed during the examination[154,168]:

1 Prehension function, including mode of grasping, posture during grasping, hindering associated movements and quality of hand motility;
2 Sitting behaviour, including the ability to sit up, sitting posture, trunk rotation and qualitative aspects;

3 Crawling behaviour, including symmetry of movements, posture of the head, coordination, variability in speed and fluency of movements;
4 Standing behaviour, including variability in standing up, posture, distance between feet, balance, trunk rotation, fluency and reaction to push;
5 Walking behaviour, including the ability to walk, fluency of trunk and leg movements, reciprocal arm swing, posture, gait width, balance, abduction of shoulders, spontaneous walking on tiptoe, variability in speed, manoeuvrability, ability to avoid objects;
6 Head, i.e. evaluation of cranial nerve functions;
7 Sensorimotor function by means of manipulation, including the assessment of muscle tone, muscle power, range of movements, intensity and threshold of deep tendon reflexes, and the extensor plantar response.

There are 94 items in the assessment. The inter-rater reliability of 20 items considered difficult to score, vary between kappa's of 0.62 and 1.00 (mean value 0.93)[154]. The findings of the Hempel assessment result in a clinical classification: presence or absence of cerebral palsy or minor neurological dysfunction (MND). The classification of definitely abnormal implies the presence of a distinct neurological syndrome, which leads to severe limitations in function and social participation, such as cerebral palsy. MND implies the presence of a functional impairment, which may be associated with some degree of disability. Examples are mild deviations in gross and fine motor function or mild abnormalities in muscle tone regulation or reflexes. Two optimality scores are calculated to quantify small neurological deviations in neurological condition. The two optimality scores are derived from 57 representative items in terms of optimality. For each item, criteria for optimality are defined[169]. The neurological optimality score (NOS) is the sum of all optimality items being scored or omitted during the investigation. The fluency score consists of the sum of 13 items of the NOS, dealing with the fluency of motor behaviour. Note that optimality is not equal to normality, since optimality has a more narrow range than normality. Up till now limited information is available regarding the concurrent and predictive validity of the Hempel assessment. The Hempel assessment has been used for the evaluation of the effect of prenatal exposure to polychlorinated biphenyls (PCBs), dioxins, and breastfeeding on neurodevelopment of healthy term infants[169-171]. These studies could demonstrate the subtle influences of PCB's and breastfeeding on neurodevelopmental outcome by means of the Hempel assessment[169-171].

1.4.2.1 Concluding remarks

To summarize, in this thesis both functional and qualitative instruments were used to assess neurological development in order to evaluate the effects of pre- and postnatal nutrition on neurodevelopmental outcome at 3 and 18 months. The strength of the evaluation of the quality of motor behaviour is the ability to assess minor neurological deviations. This qualitative approach is employed for evaluating the subtle influences of pre- and postnatal nutrition on neurodevelopmental outcome of healthy term infants.

1.5 Specific questions addressed in the thesis

This thesis has been divided into two main parts. The first part reports the results of the double blind randomized trial regarding the effect of LCPUFA-supplemented formula feeding on neurodevelopmental outcome of healthy term infants at 3 and 18 months. See for a general outline of the LCP-project see figure 5, page 50. The following research questions will be evaluated:

- Is postnatal LCPUFA supplementation for two months beneficial for neurodevelopment as derived from the quality of general movements at the age of 3 months?
- Has the duration of breastfeeding a beneficial effect on the quality of general movements at the age of 3 months?
- Is LCPUFA supplementation for two months beneficial for neurodevelopmental outcome at 18 months of age as derived from the Hempel neurological examination and the Bayley Scales?

The second part will focus on the relationship between neonatal fatty acid status and neurodevelopmental outcome at 3 and 18 months. The following specific research questions will be addressed:

- Are there differences in prenatal fatty acid status between infants exhibiting normal and mildly abnormal general movements at 3 months?
- Is there an association between prenatal fatty acid (especially LCPUFAs and *trans* fatty acid) status on the one hand and the neurological optimality score and the Bayley scales at 18 months of age on the other hand?

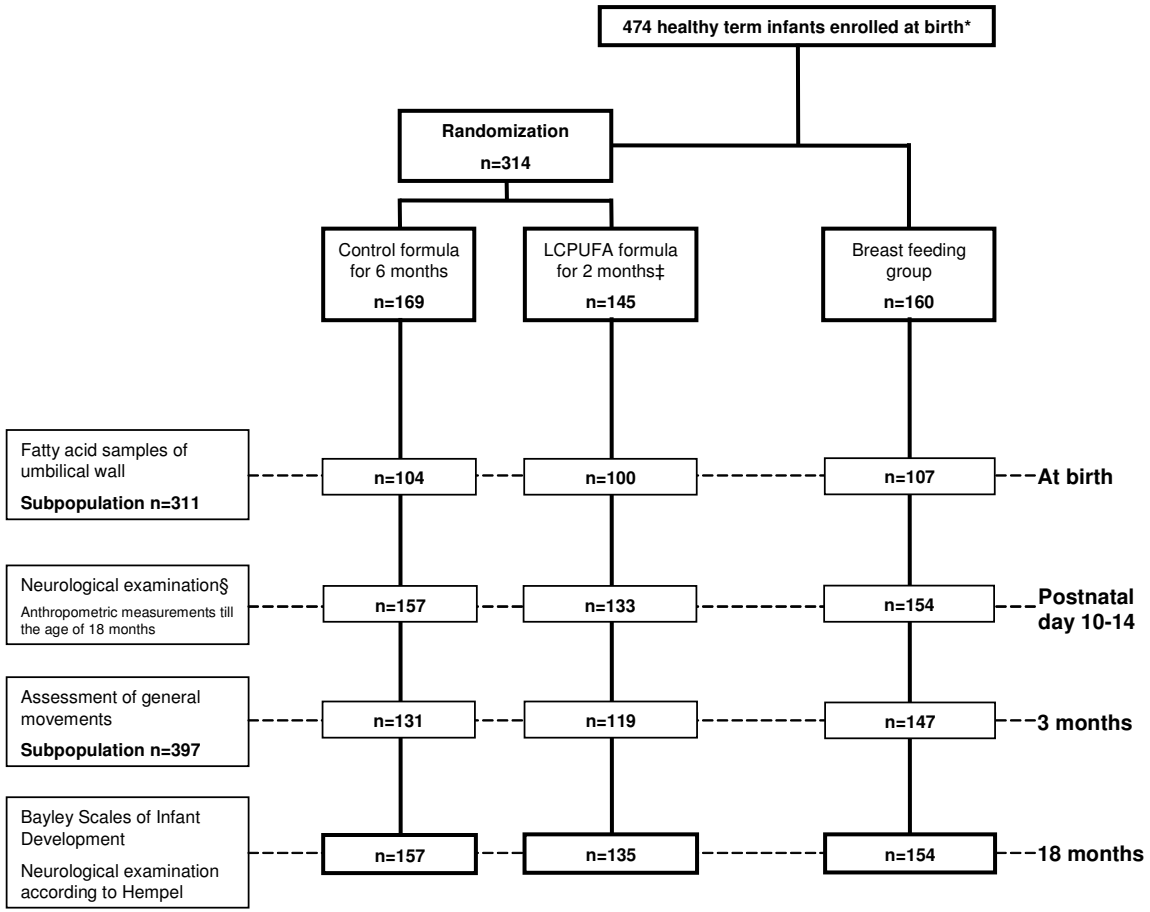

Figure 5. Study design of the LCP-project. *Two of the 474 infants met the exclusion criteria and were excluded from analyses. ‡After 2 months the infants received control formula till the age of 6 months. §The relationships between umbilical fatty acid content and the neurological examination at postnatal day 10-14 are published elsewhere by Dijck-Brouwer et al. 2005.

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Effects of early postnatal feeding on neurodevelopment

2

2.1

Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants

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American Journal of Clinical Nutrition 2003;78:313-8

Abstract

Background: Whether long-chain polyunsaturated fatty acids (LCPUFAs) play a role in the development of the young nervous system in term infants is debated.

Objective: We investigated whether supplementation of formula with LCPUFAs for 2 months improves the quality of general movements (GMs) in healthy term infants at 3 months of age.

Design: A prospective, double-blind, randomized controlled study was conducted with 2 groups of healthy term infants: a control formula (CF) group (n = 131) and an LCPUFA-supplemented-formula (LF) group (n = 119). A breastfed (BF) group (n = 147) served as a reference. Information on potential confounders was collected at enrollment. Videotapes were made of the infants' spontaneous motor behavior at 3 months of age to assess the quality of their GMs. On the basis of quality, normal GMs were classified as normal-optimal or normal-suboptimal, and abnormal GMs were classified as mildly or definitely abnormal. Attrition at 3 months of age was 15% and non-selective. Multivariate regression analyses with adjustment for confounders were carried out to evaluate the effect of the type of feeding.

Results: None of the infants had definitely abnormal GMs. Infants in the CF group had mildly abnormal GMs significantly more often than did infants in the LF and BF groups (31% compared with 19% and 20%, respectively). Infants in the BF group had normal-optimal GMs more frequently than did infants in the LF and CF groups (34% compared with 18% and 21%, respectively). Logistic regression analyses confirmed these findings.

Conclusion: Supplementation of healthy term infants with LCPUFAs during the first 2 months of life reduces the occurrence of mildly abnormal GMs.

Introduction

Fatty acids and especially long-chain polyunsaturated fatty acids (LCPUFAs) have become a major focus of attention in the field of infant nutrition. The most important LCPUFAs are docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6). During the first postnatal weeks, newborns do not seem to synthesize sufficient amounts of LCPUFAs from their precursors to satisfy the newborns' high needs (1). Infants obtain LCPUFAs from breast milk but generally not from formula. Because animal studies indicated that LCPUFAs play an important role in the development of the nervous system (2), the question arose whether infant formulas should be supplemented with LCPUFAs. Yet, no convincing evidence has been provided that LCPUFA supplementation in full-term infants confers a benefit for visual, motor, and cognitive development that extends beyond the first year of life (3). However, studies that follow children until they reach school age have not been carried out. When evaluating the effect of supplementation of infant formulas, it should be kept in mind that the putative positive effects are at best only subtle. It can be assumed that the composition of the optimal infant formula is such that it results in a developmental outcome that is similar to that observed in breastfed children. Studies that evaluated the differences in cognitive outcome between children who had been breastfed and those who received formula without LCPUFAs showed that the long-term advantage of the breastfed children was ≈ 3 to 6 intelligence quotient points (4, 5). This means that at best a possible positive effect of LCPUFA supplementation of infant formulas is on the order of a few intelligence quotient points. Until now, studies that dealt with the effect of LCPUFA supplementation on infant motor and cognitive development frequently used the Bayley Scales of Infant Development or the Fagan Test. The Bayley Scales are a frequently used but rather gross instrument to document infants' motor and cognitive abilities. The Fagan Test is supposed to be a specific cognitive test that evaluates infants' interest in novelty. Putative positive effects of LCPUFAs may be found when more sensitive and specific assessment techniques for infant development are used (2). The evaluation of the quality of general movements (GMs) has been shown to be an accurate technique for evaluating the quality of brain function in young infants (6–8). GMs are complex movements that involve the head, trunk, arms, and legs. GMs arise during early fetal life and persist until 3–4 mo after term age. Normal GMs are characterized by fluency, variation, and complexity. These characteristics disappear when movements become abnormal. Movement fluency is the first property to disappear, which means that subtle dysfunctions of the nervous system already result in movements with a jerky or stiff appearance. Movement complexity and movement variation, which in fact can be considered as 2 forms of movement variation, are the major characteristics of GM quality. Four classes of GM quality can be distinguished: 2 forms of normal GMs (normal-optimal and normal-suboptimal) and 2 forms of abnormal GMs (mildly and definitely abnormal) (**Table 1**). Various studies have shown that the quality of GMs is a strong predictor of

TABLE 1. Classification of the quality of general movements[1]

Classification	Complexity[2]	Variation[3]	Fluency[4]
Normal-optimal	+++	+++	+
Normal-suboptimal	++	++	−
Mildly abnormal	+	+	−
Definitely abnormal	−	−	−

[1] From reference 11. +++, abundantly present; ++, sufficiently present; +, present, but insufficiently; -, absent.
[2] Defined as spatial variation. The infant actively produces frequent changes in the direction of movement of the participating body parts. The changes in direction are brought about by continuously varying combinations of flexion-extension, abduction-adduction, and endorotation-exorotation of the participating joints.
[3] Defined as temporal variation. Across time, the infant produces continuously new patterns of movement, ie, the infant has an apparently infinite movement repertoire.
[4] Defined as the presence of smooth, supple, and graceful movements. Fluency in particular points to the velocity profile of the movements, which is characterized by gradual accelerations and decelerations.

neurodevelopment and that the best predictions are those that are based on the quality of movements during the last phase of GMs (9, 10). The last phase of GMs occurs at 2–4 mo after term and consists of so-called "fidgety" GMs, during which movement complexity, variation, and fluency are expressed particularly in tiny and elegant movements that occur all over the body. The occurrence of definitely abnormal GMs at the fidgety age predicts the development of cerebral palsy with a high accuracy (9, 10). The occurrence of mildly abnormal GMs at 2–4 mo of age is associated with a significant increase in the risk of development of minor neurologic dysfunction, attention problems, and aggressive behavior at school age (10). A recent study showed that in children without cerebral palsy, the quality of GMs at the fidgety age as classified according to the 4 classes shown in Table 1 was significantly correlated with neurologic development at 1.5 y of age ($\rho = 0.55$, $P < 0.001$) and at 5.5 y of age ($\rho = 0.32$, $P < 0.05$) (11). The aim of the present study was to assess the effect of LCPUFA supplementation of healthy term infants until the age of 2 mo on their neurologic condition at 3 mo, which was determined by evaluating the quality of their GMs. To this end, we videotaped the GMs of 119 infants who were fed formula supplemented with LCPUFAs and of 131 infants who were fed formula without LCPUFAs. To assess whether infants who were fed LCPUFA-supplemented formula would perform similarly to breastfed infants, we also videotaped the GMs of 147 infants who had been breastfed.

Subjects and methods

From February 1997 until October 1999, 472 healthy term infants were enrolled in the study. Mother-infant pairs were recruited during pregnancy checkup visits at the various study subsites in and near Groningen, which were located at the University and Martini Hospitals in Groningen and at midwife clinics. Final enrolment in the study occurred in the

neonatal period, at which time the parents provided written informed consent. All infants were born at 37–42 wk of gestation and were of native western European origin. We excluded from the study infants who had a congenital disorder that interfered with adequate functioning in daily life, infants from multiple births, infants whose mothers did not have mastery of the Dutch language or suffered from significant illness or disability, adopted and foster infants, and formula-fed infants who had received human milk for >5 d. We aimed to have 3 groups of comparable size: 2 groups of formula fed infants and 1 group of breastfed infants. After the mothers chose to either breastfeed or formula-feed their infants, the formula fed infants were randomly allocated to either the control-formula (CF) group or the LCPUFA-supplemented-formula (LF) group by means of a single, central computerized randomization that used a block design (blocks of 6, delivered in batches of 78). Number identification linked specific batches of formula to the infants. Accordingly, the CF group consisted of 167 newborns, the LF group consisted of 145 newborns, and the breastfed (BF) group consisted of 160 newborns. The study diets consisted of commercial formula (Nutrilon Premium; Nutricia, Zoetermeer, Netherlands) for the CF group and of a similar formula enriched with 0.45% (by wt) AA and 0.30% (by wt) DHA for the LF group. DHA was derived from egg yolk and tuna oil that was low in eicosapentaenoic acid, and the source of AA was egg yolk and a singlecell oil produced by a common soil fungus, *Mortierella alpina*. Care was taken to provide the LCPUFAs in a ratio of phospholipids to triacylglycerol that was similar to that present in human milk. The fatty acid compositions of the study formulas and of human breast milk from a comparable Dutch reference group are provided in **Table 2**. The duration of supplementation was 2 mo. Seventy-three infants in the BF group stopped breastfeeding before 2 mo of age and received LCPUFA-supplemented formula for the duration of the 2-mo period; the median duration of LCPUFA supplementation in these infants was 3 wk. All the formula-fed infants received control formula from 2 to 6 mo of age. Compliance with the specific forms of feeding was confirmed by checking the daily diaries filled out by the mothers. The formulas were provided free of charge to the parents. The parents and the examiners were unaware of the type of formula that the infants received. The study was approved by the Ethics committee of the Groningen University Hospital (MEC 95/08/207). At enrollment, detailed and standardized information was collected on the infants' social and pre- and perinatal conditions. For the latter, we used the 74 variables of the Obstetrical Optimality Score (OOS), which describes the obstetric condition, ranging from the parents' socioeconomic status and health condition to the infant's condition immediately after birth. The number of items having a value within a predefined optimal range forms the optimality score for an infant (13). We used the information obtained from the OOS both as raw data and as data dichotomized into optimal and nonoptimal categories. Follow-up at the age of 3 mo was performed for 397 infants (ie, 84% of the original population of 472 infants). The major reason that infants were not followed up was simply an overload of work for the research team (**Figure 1**). The social and pre- and perinatal background of the infants who were not

included in the assessment at 3 mo of age did not differ significantly from that of the originally recruited sample. Relevant data on the obstetric, physical, and social characteristics of the 3 groups who were assessed at 3 mo of age are provided in **Table 3**.

TABLE 2. Fatty acid composition of the study formulas and human breast milk from a comparable Dutch reference group[1]

Fatty acids (mol/100 mol)	Reference BF	LF	CF
Saturated			
C6:0	0.32 ± 0.04	0.21	0.38
C8:0	0.66 ± 0.10	3.80	2.88
C10:0	2.67 ±0.54	2.65	1.90
C12:0	8.16 ±2.60	10.78	11.46
C14:0	8.01 ± 1.98	4.53	4.50
C16:0	23.04 ± 2.19	20.03	22.72
C18:0	7.25 ± 0.92	3.85	3.29
C20:0		0.34	0.33
C22:0		0.23	0.23
Monounsaturated			
C16:1 (n-7)		0.21	0.20
C18:1 (n-9)		37.46	38.95
C20:1 (n-9)		0.25	0.25
Polyunsaturated			
C18:2 (n-6)	13.62 ± 4.24	11.0	11.56
C18:3 (n-6)	0.11 ± 0.03	0.18	-
C20:3 (n-6)	0.34 ± 0.06	0.03	-
C20:4 (n-6)	0.34 ± 0.06	0.39	-
C18:3 (n-3)	1.11 ± 0.35	1.30	1.27
C20:5 (n-3)	0.06 ± 0.04	0.06	-
C22:6 (n-3)	0.19 ± 0.11	0.23	-
Other fatty acids		1.53	

[1]LF, formula supplemented with long-chain polyunsaturated fatty Acids; CF, control formula (Nutrilon Premium; Nutricia, Zoetermeer, Netherlands).

[2]Values form reference 12.

[3]Mean ± SD.

TABLE 3. Obstetrical, physical, and social characteristics of the 3 groups who were assessed at 3 mo of age[1]

Variable	CF-group (n = 131)	LF-group (n = 119)	BF-group (n = 147)
Male Gender (%)	72 (55)	63 (53)	75 (51)
Gestational age (wk)	39.6 ± 1.2[2]	39.6 ± 1.3	39.7 ± 1.3
Post-conceptional age (wk)	54.4 ± 2.2	54.6 ± 2.8	53.4 ± 2.2[3,4]
Birth weight (g)	3514 ± 430	3534 ± 502	3592 ± 424
First born [*n* (%)]	51 (39)	48 (40)	71 (48)
Maternal age (y)	30 ± 4	30 ± 4	31 ± 5[3]
Maternal higher education[5] [*n* (%)]	8 (6)	19 (16)	61(42)[3,4]
Paternal higher education[5] [*n* (%)]	18 (14)	19 (16)	62 (42)[3,4]
Maternal smoking during pregnancy [*n* (%)]	42 (32)	38 (32)	28 (19)[3,4]
Paternal smoking during pregnancy [*n* (%)]	60 (46)	63 (53)	53 (36)[3,4]
Maternal alcohol consumption during pregnancy [*n* (%)]	13 (10)	13 (11)	38 (26)[3,4]
Obstetrical Optimality Score[6]	57, 59, 65	56, 58, 65	57, 60, 66
Weight at 3 mo of age (g)	6325 ± 714	6410 ± 714	6266 ± 746
Length at 3 mo of age (cm)	63 ± 2.2	63 ± 2.6	63 ± 2.5

[1]CF, control formula; LF, formula supplemented with long-chain polyunsaturated fatty acids; BF, breastfed.
[2]Mean ± SD.
[3]Significantly different from the CF group, $P < 0.05$ (Bonferroni correction).
[4]Significantly different from the LF group, $P < 0.05$ (Bonferroni correction).
[5]University education or vocational college.
[6]25th, 50th, and 95th percentiles.

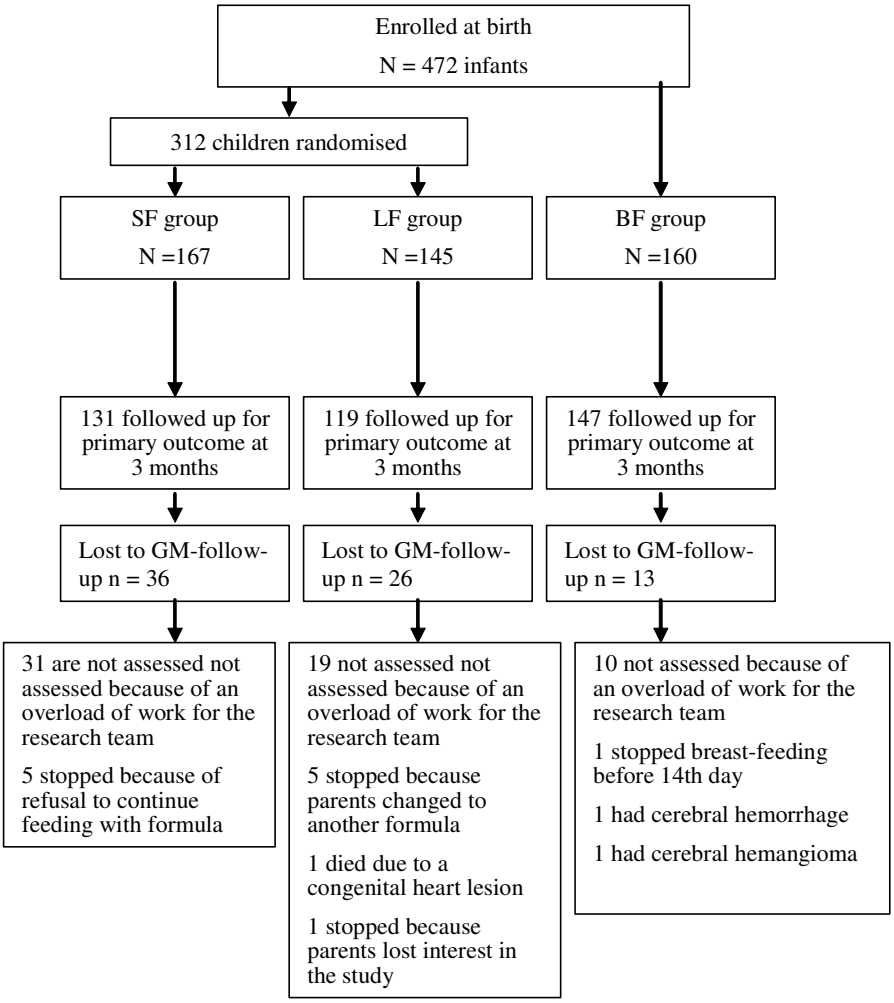

FIGURE 1. Flow diagram of infants enrolled in the study and followed up until 3 mo of age. CF, control formula; LF, formula supplemented with long-chain polyunsaturated fatty acids; BF, breastfed; GM, general movements.

The follow-up at 3 mo of age consisted of videotaping the infants' spontaneous motility for 15 min while they were in the supine position and in their home environment. Care was taken to ensure that the infants were awake, active, and not crying. At follow-up, the infants had a postmenstrual age of ≥ 49 wk; thus, all the infants were assessed in the final phase of GMs. Investigators who were blinded to the subjects' group assignments analyzed the quality of the videotaped GMs. Movements were classified as normal-optimal, normal-suboptimal, mildly abnormal, and definitely abnormal (Table 1; 11). Interscorer agreement on GM quality, which was determined in a random sample of 10 videotapes, was good (K= 0.75; 14). At the time of follow-up, the infants' weight and length were recorded (Table 3).

The analysis focused on the effect of type of feeding on GM quality. Besides univariate statistical analyses with chi-square, logistic regression analysis was applied because this offered the possibility of parceling out the effect of type of feeding on movement quality while taking into account the role of potential confounders (15). For calculating the effect of type of feeding, a dummy variable was created for the intake of each of the 3 diets (ie, CF, LF, and breast milk). Two runs of logistic regression analysis were carried out: one for the contribution of type of feeding to the occurrence of normal-optimal GMs and one for the effect of type of feeding on the occurrence of mildly abnormal GMs. Other factors included in the multivariate analyses were social characteristics (*see* Table 3), postconceptional age, OOS, and anthropometric variables. In addition, we used logistic regression analysis to evaluate whether the duration of LCPUFA supplementation in the BF group played a role in the development of mildly abnormal and normal optimal GMs. Statistical calculations were performed with SPSS version 10 (SPSS Inc, Chicago). Differences having a P value < 0.05 were considered statistically significant (two-tailed testing).

Results

The distribution of the quality of GMs at 3 mo of age in the 3 groups is depicted in **Figure 2**. None of the infants had definitely abnormal GMs, and ≈ 20–30% of the infants had mildly abnormal GMs. The frequency of mildly abnormal GMs was significantly higher in the CF group than in the LF group (31% compared with 19%; $P = 0.04$). Normal-optimal GMs tended to occur most frequently in the BF group (34% compared with 18% and 21% in the LF and CF groups, respectively), but these differences were not significant in the univariate analyses. A summary of the results of logistic regression analysis of factors contributing to the occurrence of mildly abnormal GMs is presented in **Table 4**. The analysis confirmed that mildly abnormal GMs occurred significantly less often in the LF group than in the CF group. Similarly, the infants in the BF group had significantly fewer mildly abnormal GMs than did the infants in the CF group. Logistic regression also confirmed that the frequency of mildly abnormal GMs did not differ significantly between the LF and BF groups. Besides including the intake of CF, the model explaining the occurrence of mildly abnormal GMs also included a family history of diabetes, a gestational age at birth of < 40 wk, a perineum at birth characterized as having undergone an episiotomy or a grade-1–2 rupture, a nonmarital state, and a young postnatal age at GM assessment. When gestational age and postnatal age at GM assessment were replaced in the logistic regression analysis by postconceptional age, the effect of type of feeding did not change significantly. An older postconceptional age was significantly related to a less frequent occurrence of mildly abnormal GMs (odds ratio: 0.85; 95% CI: 0.78, 0.99; cf

Table 4). A summary of the results of logistic regression analysis of factors contributing to the occurrence of normal-optimal GMs is shown in **Table 5**.

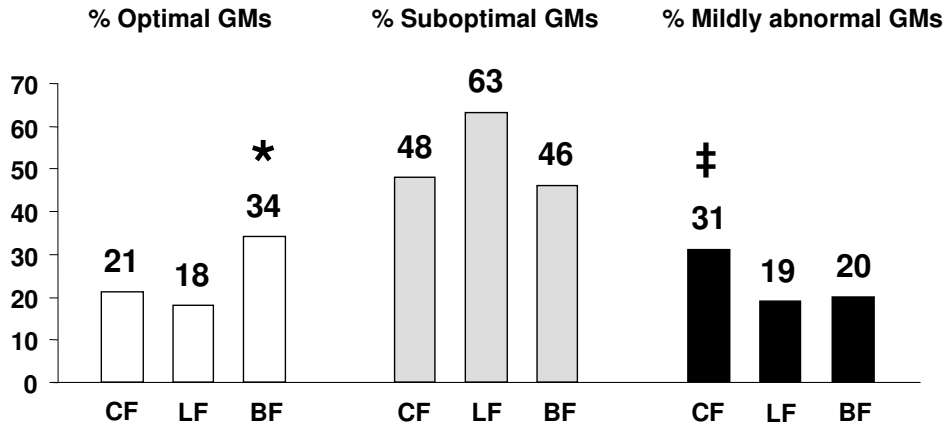

FIGURE 2. The distribution of the quality of general movements (GMs) in each feeding group at 3 months. CF = control formula; LF = LCPUFA supplemented formula group; BF = reference breastfeeding group. No definitely abnormal GMs were observed in this population. *Significantly more optimal GMs in the breastfeeding group compared with formula groups in the multivariate analysis. ‡Significantly less mildly abnormal GMs in the LCPUFA supplemented formula group compared with the control formula group ($p<0.05$).

The analysis indicated that breastfeeding was associated with a significantly higher prevalence of normaloptimal GMs than was CF or LF feeding. Besides including the intake of breast milk, the model explaining the occurrence of normal-optimal GMs included a profession of the mother's partner that required a university or vocational-college education, a high OOS, and an old age at GM assessment. In addition, the replacement of gestational age and postnatal age at GM assessment by postconceptional age did not significantly change the effect of type of feeding on the occurrence of normal-optimal GMs. Postconceptional age had no significant effect on normaloptimal GMs. Logistic regression analysis in the BF group showed that the duration of LCPUFA supplementation did not significantly affect movement quality.

TABLE 4. Results of logistic regression analysis of factors contributing to the occurrence of mildly abnormal general movements (explained variance of 9.9%)[1]

Factors	Odds ratio (95% CI)	P
Type of feeding		
Breast milk	1	
CF	2.03 (1.09, 3.80)	0.039
LF	0.94 (0.48, 1.85)	0.87
LF[2]	0.49 (0.26, 0.92)	0.032
Covariates		
Marital state[3]	0.57 (0.32, 1.01)	0.039
Family history of diabetes[4]	1.86 (1.09, 3.18)	0.011
Gestational age at birth[5] (wk)	0.40 (0.21, 0.76)	0.010
Condition of perineum[6]	2.44 (1.38, 4.33)	0.002
Age at assessment (wk)	0.86 (0.76, 0.98)	0.021

[1]CF, control formula; LF, formula supplemented with long-chain polyunsaturated fatty acids.
[2]With the CF group instead of the breastfed group as the reference group.
[3]0 = not married, 1 = married.
[4]One of the variables of the Obstetrical Optimality Score, denotes the presence (1) or absence (0) of type 1 or type 2 diabetes in ≥1 first-degree relative.
[5]0 = <40 wk, 1 = ≥40 wk.
[6]0 = episiotomy or grade-1–2 rupture, 1 = intact perineum or total rupture.

TABLE 5. Results of logistic regression analyis of factors contributing to the occurrence of normal-optimal general movements (explained variance of 4.6%)[1]

Factors	Odds ratio (95% CI)	P
Type of feeding		
Breast milk	1	
CF	0.55 (0.31, 0.97)	0.038
LF	0.42 (0.23, 0.78)	0.006
LF[2]	0.77 (0.40, 1.45)	0.41
Covariates		
Profession of mother's partner required a university or vocational college education[3]	1.70 (0.99, 2.92)	0.055
Obstetrical Optimality Score	1.05 (0.99, 1.12)	0.11
Age at assessment (wk)	1.08 (0.97, 1.21)	0.17

[1]CF, control formula; LF, formula supplemented with long-chain polyunsaturated fatty acids.
[2]With the CF group instead of the breastfed group as the reference group.
[3]0 = no, 1 = yes.

Discussion

Our study indicated that supplementation of formula with LCPUFAs for 2 mo after birth significantly reduces the occurrence of mildly abnormal GMs at the age of 3 mo. We showed this effect in a relatively large series of full-term, healthy infants, in whom the quality of motor behavior was assessed in a double-blind way. Fifteen percent of the original sample was lost to follow-up, but the loss was nonselective. At an early age, the nervous system is organized basically in a non-specific, generalized way (16). The GMs, which are the most frequently occurring movements until the age of 3–4 mo, are an expression of this organization. Various studies support the notion that the quality of GMs reflects the quality of the nervous system (7–11, 14). The finding that LCPUFA supplementation may induce an improvement in GM quality fits with the evidence that LCPUFA accretion in early life occurs in all cortical areas, where it might play a major role in the formation of synapses (17). It has been suggested that DHA affects synapse formation directly by means of membrane incorporation, whereas the role of AA in synapse formation is more indirect because it particularly affects signal transduction events that regulate growth cone activity and synapse formation (1). The occurrence of mildly abnormal GMs at 3 mo of age indicates an increased vulnerability of the brain to the development of so-called minor developmental disorders, such as minor neurologic dysfunction, clumsiness, and attention problems at school age (10, 11). Thus, the finding that LCPUFA supplementation is associated with a decrease in mildly abnormal GMs may imply that LCPUFAs have a protective effect on the development of minor developmental disorders. Yet, the beneficial effect of LCPUFA supplementation on neurologic condition could be a temporary one. Such a temporary positive effect was reported by some researchers for the effect of LCPUFAs on visual function (18, 19) and psychomotor development (20, 21), but other researchers found no significant effects (22, 23). Whether or not LCPUFAs have a long-term beneficial effect on brain function in full-term infants is still debated. Birch et al (24) reported a positive effect on visual and mental development but not on motor development at 18 mo of age. Yet, several studies did not find an effect of LCPUFAs on visual (22) or psychomotor (23, 25, 26) development at 1–2 y of age. Whether LCPUFA supplementation affects the long-term developmental outcome of term infants can be determined only on the basis of follow-up studies that apply sensitive and specific tools for evaluating brain function at school age. In the present study, the GM quality of the infants in the BF group was significantly higher than that of the infants in the CF and LF groups. The higher GM quality of the infants in the BF group was not related to the duration of LCPUFA supplementation provided during part of the 2-mo feeding period. The breastfed infants' better performance is in agreement with the results of the literature, which indicate that the cognitive and motor development of breastfed infants is better than that of formula-fed infants (4, 5, 27). However, we cannot exclude the possibility that this

result is explained by factors other than the composition of breast milk, such as maternal hormones that are not present in formula, subtle differences in care giving practices (bonding), and genetic differences between mothers who breastfeed and those who bottle-feed (28). The logistic regression analysis indicated that their better socioeconomic background did not explain the breastfed infants' better performance. In conclusion, the present study indicates that LCPUFA supplementation of healthy term infants for 2 mo improves their neurologic condition at 3 mo of age. Whether the LCPUFA-induced advantage is transient or permanent needs to be determined.

Acknowledgements

We kindly acknowledge the statistical advice of V Fidler. ERB and MH-A designed the study; HB, DAJD-B, ERB, FAJM, and MH-A analyzed and interpreted the results and wrote the report; JALW and HMT collected the video footage, participated in analyzing the videotapes, and were involved in the writing process; and JCvdH carried out the major part of analyzing the videotapes and was involved in the writing of the report. None of the authors had any conflicts of interest.

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2.2

Exclusive breastfeeding of healthy term infants for more than 6 weeks improves neurological condition

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Journal of Nutrition 2003;133:4243-5

Abstract

To investigate the minimal duration of exclusive breastfeeding for optimal neurological outcome, we assessed the quality of general movements (GM) at 3 mo of 147 breastfed healthy term infants that were followed from birth. The quality of GM is a sensitive marker of neurological condition. The quality of GM was classified as normal-optimal, normal-suboptimal, mildly abnormal and definitely abnormal. Information on social and pre- and perinatal conditions and the duration of breastfeeding was collected prospectively. Logistical regression analyses were used to adjust for confounders. There was a positive association between breastfeeding duration and movement quality, with a saturation effect at the age of ≈6 wk. In the group of infants breastfed for ≤ 6wk (n = 55), 18% exhibited normal-optimal GM, 47% normal-suboptimal GM, and 47% mildly abnormal GM. In contrast, in the group of infants breastfed for > 6 wk (n = 92), 43% exhibited normal-optimal GM, 45% normal-suboptimal GM, and 12% mildly abnormal GM. Exclusive breastfeeding for >6 wk was therefore associated with markedly less abnormal and more normal-optimal GM. Thus, we conclude that breastfeeding for >6 wk might improve the neurological condition in infants.

Introduction

In general, breastfed infants show better cognitive development than formula-fed infants (1). In addition, cognitive function improves with the duration of breastfeeding (1). Whether the same holds true for neurological condition is uncertain. Lanting et al. (2) retrospectively collected information on the duration of breastfeeding in a mixed population of infants with and without risk for developmental disorders that were followed from birth. They reported that breastfeeding for ≥ 3 wk was associated with better neurological condition at 9 y than formula feeding (2). In a prospective study, Lanting et al. (3) indicated that children exclusively breastfed for ≥ 6 wk moved more fluently at 3.5 y than did formula-fed age matched controls. However, the Lanting studies did not address the question of the minimum duration of exclusive breastfeeding needed to achieve optimal neurological condition. The present paper addresses this question. We recently conducted a double-blind randomized trial on the effect of feeding formula supplemented with long-chain PUFA (LCPUFA)[3] on the quality of general movements (GM) in healthy term infants (4). The quality of GM is a recently developed sensitive instrument for the assessment of brain function in young infants (5,6). General movements are spontaneous movements of the young infant involving all parts of the body. Normal GM are characterized by variation, complexity and fluency. These characteristics disappear when movements become abnormal. Movement fluency is the first property to disappear, indicating that subtle dysfunctions of the nervous system already cause jerky or stiff movement at this early phase of development. Movement complexity and movement variation, which in fact can be considered as two forms of variation, are the major characteristics of GM quality. Four classes of GM-quality can be distinguished: two forms of normal GM (normal-optimal and normal-suboptimal) and two forms of abnormal GM (mildly and definitely abnormal). The quality of GM at the age of ≈ 3 mo is a powerful predictor of neurological outcome. Definitely abnormal GM at 3 mo predicts the development of cerebral palsy with an accuracy of 85 to 98%, whereas the presence of mildly abnormal GM at 3 mo is associated with increased risk of minor neurological dysfunction and attention problems at school age (5–7). Bouwstra et al. (4) studied three groups of newborn infants (n = 397) for the first 2 mo; 119 infants were fed formula supplemented with LCPUFA, 131 infants were fed control formula and 147 infants were breastfed. Infants fed control formula markedly more often exhibited mildly abnormal GM than did the infants of the other two groups (31% vs 19 to 20%). Thus, LCPUFA supplementation had a beneficial effect on the quality of GM. The breastfed infants (the reference group) more often exhibited normal-optimal GM (34% vs. 18 to 21%). The present report examines the breastfed reference group only and aims to determine the minimum duration of breastfeeding needed to achieve optimal GM quality.

Materials and Methods

The infants observed in the present study also participated in the previously mentioned study of Bouwstra et al. (4). The study was approved by the Medical Ethics Committee of the Groningen University Hospital. Healthy full-term breastfed infants (n = 160) were enrolled at birth, at which time the parents gave informed consent. At enrollment detailed information was collected on a wide range of social and pre- and perinatal conditions. Obstetrical condition was measured by the 74 variables of the Obstetrical Optimality Score (OOS) (8). Information on the duration of breast-feeding was collected prospectively. When breastfeeding stopped before the age of 2 mo, infants were fed formula supplemented with LCPUFA until 2 mo (n = 73). Follow-up at 3 mo (13.8 +/- 1.4 wk postnatal age) was achieved with 147 infants (i.e., 92% of the original population). Researchers took care to assess all infants during the last phase of GM development, during which GM quality is relatively stable. The dropouts were non selective. Follow-up consisted of a 15-min video recording of spontaneous mobility in the supine position. Using blind study procedures, investigators assigned these recordings to four GM categories (9): normal-optimal (rich variation and complexity, fluent), normal-suboptimal (sufficiently variable and complex, not fluent), mildly abnormal (poor variation and complexity, not fluent) and definitely abnormal (virtually no variation, complexity or fluency). Interscorer agreement on GM quality, determined with a random sample of 10 videos, was good [κ = 0.75; (10)].

Statistical methods

Statistical analyses focused on the effect of duration of exclusive breastfeeding on the presence of mildly abnormal GM and normal-optimal GM. In addition to univariate statistical analysis with χ^2 or Fisher's exact test, two runs of binary logistical regression analysis were carried out to adjust for potential confounders, one analyzing the effect of the duration of exclusive breastfeeding on the presence of normal-optimal GM and the other analyzing the effect of the duration of breastfeeding on the occurrence of mildly abnormal GM. Variables describing the social condition (see **Table 1**) and the variables of the OOS were also included in the multivariate analysis. In the univariate analyses of the relationship between duration of exclusive breastfeeding and quality of GM, P-values of <0.0167 (Bonferroni correction: 0.05/3; two tailed) were considered significant; in the other analyses P-values of 0.025 (two tailed) were considered significant.

TABLE 1. Obstetrical and social characteristics of the two breastfed groups[1]

Variables	≤ 6 wk exclusive BF[2]	> 6 wk exclusive BF	P-value
Infants, *n*	55	92	
Males, %	53	50	NS[3]
Birth weight, g	3580 ± 418	3599 ± 429	NS
First born, %	53	46	NS
Maternal age, y	30 ± 4.4	32 ± 5.1	NS
Maternal higher education,[4] %	26	51	<0.0005
Maternal professional status,[5] %	20	40	<0.0005
Maternal alcohol consumption during pregnancy, %	13	34	<0.0005
Maternal smoking during pregnancy, %	22	17	NS
Paternal higher education,[4] %	35	47	0.005
Paternal smoking during pregnancy, %	49	28	0.001
Paternal professional status,[5] %	38	51	0.024
OOS[6]	58 ± 4.7	60 ± 3.3	NS
Postnatal age at assessment, wk	13.9 ± 1.6	13.7 ± 1.3	NS

[1]Values are means ± SD or %. Dichotomized values were determined by χ^2 analysis, continuous values by t-test.
[2]BF, breastfeeding.
[3]NS, nonsignificant (P < 0.025).
[4]University or vocational college education.
[5]Member of a profession requiring a university or vocational college education, or an entrepreneur.
[6]OOS, Obstetrical Optimality Score consisting of 74 items.

TABLE 2. Distribution of general movements (GM) quality at various cut-off points of duration of exclusive breastfeeding

Cut-off Wk	*n*	Normal-optimal GM, % (*n*)	P	Normal-suboptimal GM, % (*n*)	Mildly abnormal GM % (*n*)	P
> 3	127	36 (46)	0.24	46 (58)	18 (23)	1.00
> 4	117	38 (45)	0.02	46 (53)	16 (19)	0.03
> 5	106	40 (42)	0.02	44 (47)	16 (17)	0.06
> 6	92	44 (40)	0.002[1]	44 (41)	12 (11)	0.001[1]
> 7	88	42 (37)	0.01	46 (40)	12 (11)	0.004[1]
> 8	74	38 (28)	0.32	48 (36)	14 (10)	0.04
> 9	70	37 (26)	0.44	49 (34)	14 (10)	0.08
>10	61	39 (24)	0.25	46 (28)	15 (9)	0.15
>11	55	40 (22)	0.24	49 (27)	11 (6)	0.03
>12	49	43 (21)	0.11	47 (23)	10 (5)	0.03

[1]Significant difference in movement quality between infants breastfed for a duration longer than the cut-off point and those breastfed for a shorter period. Bonferroni correction [i.e., P < 0.0167 (0.05/3)].

Results

Infants were exclusively breastfed for a median duration of 9 wk (range 1 to 52 wk). There was a positive association between breastfeeding duration and movement quality, with a saturation effect at the age of ≈ 6 wk. Quality of GM was significantly better in infants breastfed for > 6 wk than in those breastfed for ≤ 6 wk (**Table 2**; **Fig. 1**). The social, but not the obstetrical, background of mothers who breastfed for > 6 wk differed from that of mothers who breastfed for a shorter period. As expected, longer breastfeeding was associated with a higher level of parental education and professional employment, less paternal smoking and greater maternal alcohol consumption (Table 1). Logistical regression analyses confirmed that breast-feeding for > 6 wk was associated with the presence of less mildly abnormal GM (P = 0.0015; explained variance 6.9%) and more normal-optimal GM (P= 0.0025; explained variance 6.8%). None of the potential confounders, including duration of LCPUFA supplementation, contributed significantly to the models.

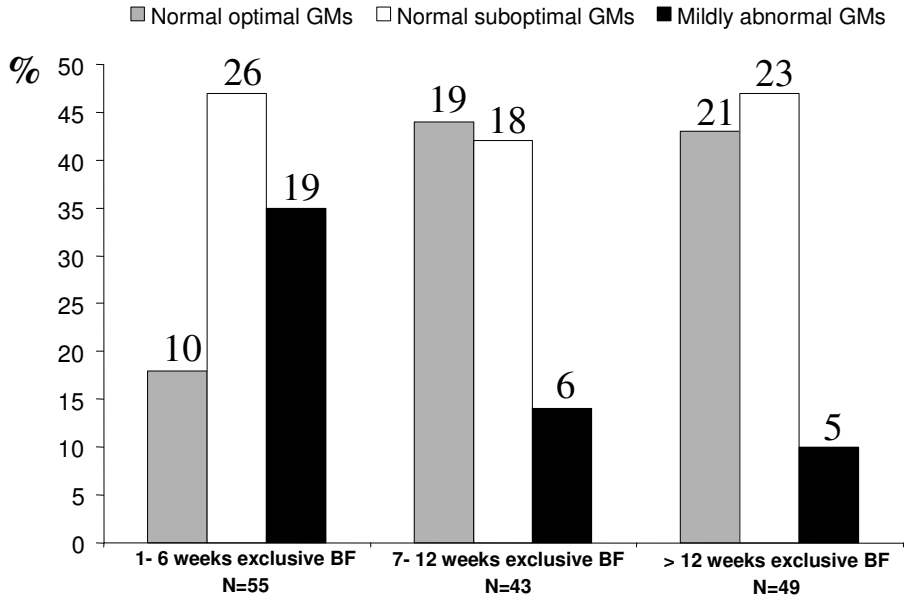

FIGURE 1. Distribution of the quality of general movements (GM) around 3 mo (13.8 ± 1.4 wk postnatal age) in groups of infants who received exclusive breastfeeding for different periods of postnatal life. Numbers above the bars represent the actual number of infants in each category. There were no infants with definitely abnormal GM. Note that the distribution of GM quality in the two groups on the right side of the figure correspond to the data of table 2 at the cut-off of > 6 wk. BF = breastfeeding.

Discussion

The results of this study indicate that exclusive breastfeeding for >6 wk is associated with a beneficial effect on the quality of GM at the age of 3 mo. This finding is of clinical relevance because the quality of GM correlates with neurobehavioral condition at school age (5,6). It is important to realize that we report an association, which does not necessarily imply causation. However, a study to demonstrate causation cannot be performed, because randomized trials on breastfeeding are not ethically justified. Three explanations can be offered for the association between longer duration of breastfeeding and better neurological condition. First, it may be that infants with better neurological condition are more likely to be breastfed for a longer duration. Second, the association could be mediated by a difference in nurturing habits between the two groups, as nurturing habits (e.g., bonding) can affect neurobehavioral development. However, it is interesting to note that Lucas and Morley (11) found that the intelligence quotient of children who had been fed human milk by nasogastric tube was 8 points higher at 8 y than that of children fed formula by nasogastric tube. Third, longer exposure to specific components of breast-milk, such as LCPUFA, may beneficially affect the rapidly developing brain. At the age of 6 wk, an important period of transition in brain function begins (7,12). This is the onset of the period in which the infant becomes a social partner (13). More important, this is the age at which the quality of GM becomes predictive, indicating that basic neurocircuitries become stabilized (5,6). Based on our finding, we hypothesize that the beneficial effect of breastfeeding continues up to the onset of this period of transition in brain function. In conclusion, our study suggests that continuation of exclusive breastfeeding for more than 6 wk improves infant neurological condition.

Acknowledgements

We kindly acknowledge the help of J.A.L. Wildeman, H. M. Tjoonk and J. C. van der Heide in video recording and analysis. Numico assisted in the design of the study and in the interpretation of the results.

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2.3

Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants

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Acta Pædiatrica 2004;94:26-32

Abstract

Aim: Previously, we found a beneficial effect of 2 mo supplementation of infant formula with long-chain polyunsaturated fatty acids (LCPUFA) on neurological condition at 3 mo in healthy term infants. The aim of the present follow-up study was to evaluate whether the effect on neurological condition persists until 18 mo.

Methods: A prospective, double-blind, randomized control study was conducted. Three groups were formed: a control (CF; n=169), an LCPUFA-supplemented (LF; n=146) and a breastfed (BF; n=159) group. Information on potential confounders was collected at enrolment. At the age of 18 mo, neurodevelopmental condition was assessed by the age-specific neurological examination of Hempel and the Bayley scales. The Hempel assessment resulted in a clinical neurological diagnosis, a total optimality score and a score on the fluency of motility. The Bayley scales resulted in mental and psychomotor developmental indices. Attrition at 18 mo was 5.5% and non-selective. Multivariate regression analyses were carried out to evaluate the effect of type of feeding while adjusting for confounders.

Results: None of the children had developed cerebral palsy and 23 (CF: n=8; LF: n=10; BF: n=5) showed minor neurological dysfunction. The groups did not show statistically significant differences in clinical neurological condition, neurological optimality score, fluency score, and the psychomotor and mental development indices. Multivariate analysis confirmed that there was no effect of type of feeding on neurological condition.

Conclusion: This study indicates that the beneficial neurodevelopmental effect of 2 mo LCPUFA supplementation in healthy term infants can not be detected at the age of 18 mo.

Introduction

Long-chain polyunsaturated fatty acids (LCPUFA) are richly present in the central nervous system, especially where signal transduction takes place. Several animal studies suggest that LCPUFA have beneficial effects on the nervous system [1]. Perinatally, infants do not seem to synthesize sufficient amounts of LCPUFA from their precursors to cover their high needs. Thus, young infants are partly dependent on dietary intake of LCPUFA [2]. Human milk often is the only dietary source of LCPUFA, because standard, commercially available formulae lack LCPUFA. LCPUFA could be one of the mediators of the positive effects of breastfeeding on neurological and cognitive development [3–5]. In a previous study, we were able to demonstrate that the addition of LCPUFA to formula feeding for a duration of 2 mo in healthy term infants improved the quality of general movements at the age of 3 mo [6]. The quality of general movements is a sensitive marker of brain function [7]. Likewise, Agostoni et al. reported a beneficial effect of LCPUFA supplementation on neurodevelopment of term infants at the age of 4 mo [8]. However, a Cochrane meta analysis revealed that no consistently positive effect of LCPUFA supplementation on neurological, cognitive and visual development could be demonstrated, which persisted beyond the first year of life [9]. The absence of effect beyond the age of 1 y can be explained in two ways. First, it is possible that LCPUFA only have a temporary effect on brain development, which does not extend beyond the age of 1 y. Second, it could be that the tests used beyond the age of 12 mo, such as the Bayley Scales of Infant Development, were not sensitive enough to detect differences in neurological outcome induced by LCPUFA. In the present study, we aimed at evaluating the effect of 2 mo LCPUFA supplementation on neurodevelopmental condition at the age of 18 mo in the groups of term infants in which we were previously able to demonstrate a beneficial effect of LCPUFA at 3 mo [6]. For this, we did not only use the traditional evaluation by means of the Bayley Scales of Infant Development, but also the sensitive neurological examination described by Hempel [10]. The Hempel assessment is a standardized assessment technique designed for the detection of minor signs of neurological dysfunction. It does not only assess traditional signs of neurological dysfunction, such as mild abnormalities in muscle tone regulation and motor milestones, but also the quality of motor behaviour. The Hempel assessment results in a clinical classification (presence or absence of cerebral palsy or minor neurological dysfunction) and two optimality scores: a total neurological optimality score and a subscore on the fluency of motility. Subtle dysfunctions of the nervous system already induce a reduction of the fluency score [11]. Specifically, we addressed the following questions: 1) Does LCPUFA supplementation during the first 2 mo after birth induce a reduction in minor neurological dysfunction (MND) and an increase
in the neurological optimality score (NOS) and the fluency score (primary outcome)? 2) Does LCPUFA supplementation during the first 2 mo after birth result in higher scores on the Bayley scales (secondary outcome)?

TABLE 1. Social and obstetrical characteristics of the three feeding groups

Variables	CF-group	LF-group	BF-group
Number of children	169	146	159
Number of children assessed			
• Neurological examination by Hempel (%)	157 (93)	135 (92)	154 (97)
• Bayley Scales of Infant Development			
MDI (%)	155 (92)	135 (92)	148 (93)
PDI (%)	149 (88)	134 (92)	144 (91)
Male Gender (%)	97 (57)	79 (54)	80 (50)
Birthweight, mean ± SD (g)	3526 ± 446	3520 ± 490	3578 ± 434
First born (%)	37%	36%	46%
Maternal age (years), mean ± SD	30 ± 4.2	30 ± 4.0	31 ± 4.7[c,d]
Maternal higher education[a] (%)	5%	14%[b]	40%[c,d]
Paternal higher education[a] (%)	14%	12%	40%[c,d]
Maternal smoking during pregnancy (%)	31%	31%	19%[c,d]
Paternal smoking during pregnancy (%)	48%	50%	37%[d]
Maternal alcohol consumption during pregnancy (%)	8%	9%	24%[c,d]
Obstetrical Optimality Score	59 ± 3.8	59 ± 4.2	59 ± 4.2

[a] University education or vocational college.
[b] LCP-supplemented group different from Control formula ($p < 0.05$, Bonferroni corrected).
[c] Breast significantly different from control formula ($p < 0.05$; Bonferroni corrected).
[d] Breast significantly different from LCP formula ($p < 0.05$; Bonferroni corrected).

Methods

The children participating in the present project belong to the longitudinal study on the effect of LCPUFA supplementation on neurodevelopmental outcome. This study has been described in detail in Bouwstra et al. [6]. In short, the procedures can be summarized as follows. Final enrolment into the study occurred in the neonatal period, at which point in time the parents provided informed consent. All 474 infants were born at term. We aimed at having three groups with a comparable size: two groups of formula-fed infants and one group of breastfed infants. After the mother's choice to either breastfeed or formula-feed her infant, formula-fed infants were randomly allocated to a control formula group or to an LCPUFA-supplemented formula group by means of a single, central computerized randomization, using block design. Number identification linked specific batches of formula to the infants. Accordingly, the control formula (CF) group consisted of 169 newborns, the LCPUFA-supplemented formula (LF) group of 146 babies and the breastfed (BF) group of 159 neonates. Study diets were commercial formula for the CF group

(Nutrilon Premium; Nutricia, Zoetermeer, The Netherlands) and a similar formula enriched with 0.45% AA and 0.30% DHA for the LF group. The duration of supplementation was 2 mo. In 73 infants of the BF group, breastfeeding stopped prior to 2 mo. These 73 infants received LCPUFA-supplemented formula until the age of 2 mo for a median duration of 3 wk. All formula-fed infants received control formula between 2 and 6 mo. Parents and examiners were unaware of the type of formula-feeding the infants received. The study was approved by the Ethics Committee of the Groningen University Hospital. At enrolment, detailed and standardized information was collected on the social and pre- and perinatal conditions. For the latter, we used the 74 variables of the Obstetrical Optimality Score (OOS), which describe the obstetrical condition, ranging from the parents' socio-economic status and health condition to the infant's condition immediately after birth. The number of items with a value within a predefined optimal range forms the optimality score of an infant [12]. We used the information of the OOS both in raw data form and in the optimal/non-optimal dichotomy of the OOS. Social condition was documented by collecting information on the parent's level of education and occupation, and by means of the Home Observation for Measurement of the Environment (HOME) inventory [13]. HOME contains 45 items clustered into six subscales: Parental Responsivity, Acceptance of Child, Organization of the Environment, Learning Materials, Parental Involvement, and Variety in Experience. Assessments were carried out in the home environment, during two separate appointments. This explains why the number of children assessed with the Bayley scales is not identical to that of the number of children who had been assessed neurologically (see Table 1). Follow-up at the age of 18 mo was about 92% for all neurological outcomes (see Table 1). The major reasons for drop-out were change of type of feeding (n=10) and loss of interest in the study (n=6). The social and obstetrical characteristics of the two randomized formula groups were identical, with the exception of a slightly higher maternal education in the LF group (Table 1). The breastfed group differed substantially in social background from the two formula groups (Table 1). This is in accordance with the reports of others [14]. The Bayley Scales of Infant Development (BSIDII) were used to assess mental and psychomotor development at the age of 18 mo [15]. The mental developmental index (MDI) and the psychomotor developmental index (PDI) were scored based on the number of items successfully completed. Since the children were not exactly 18 mo of age at the time of the assessment, we converted the scores into age normalized values, as derived from recently developed Dutch norms [16]. The MDI index assesses memory, problem solving, discrimination, classification, language and social skills. The PDI index measures control of gross and fine muscle groups, including walking, running, jumping, prehension and imitation of hand movements. In addition, each child was assessed neurologically with the help of the technique described by Hempel [10]. This instrument measures, in a standardized, free-field situation, motor functions (grasping, sitting, crawling, standing and walking) of the child. In addition, muscle tone, reflexes and the function of the cranial nerves are assessed. Each toddler was classified as neurologically normal, showing signs of

minor neurological dysfunction (MND), or as definitely abnormal. The classification of definitely abnormal implies the presence of a distinct neurological syndrome, which leads to severe limitations in function and social participation, such as cerebral palsy. MND implies the presence of a functional impairment which may be associated with some degree of disability. Examples are mild deviations in gross and fine motor function or mild abnormalities in muscle tone regulation or reflexes. Besides the classification into distinct categories, we used the optimality concept to summarize the neurological condition. For 57 items representing the entire neurological examination, an optimal range was defined. The total number of items with a value within the predefined optimal range formed the neurological optimality score of an infant. It should be realized that there is a conceptual difference between normality and optimality, as the range for optimal behaviour is narrower than that of normal behaviour [17]. Due to the latter characteristic, the neurological optimality score (NOS) is an excellent instrument to evaluate subtle deviations in neurodevelopmental outcome. In addition to the total neurological optimality score, the fluency subscore was calculated. The latter score, which consists of the 13 items of the neurological optimality score, dealing with fluency of motor behaviour during various activities, is the part of the neurological optimality score which is most easily affected by subtle neurological dysfunction [11].

Statistics

Power analysis revealed that, with the present sample sizes of the groups, it would be possible to detect a mean difference in the fluency score of 0.66 or greater with a probability of 80% at the predetermined level of a=0.05, assuming a standard deviation of 1.7 [11]. With respect to our secondary outcome parameters, the power analysis indicated that, with the present sample sizes, it would be possible to detect a mean difference in the Bayley PDI and MDI of 5 points or more with a probability of 80% at the predetermined level of a=0.05, assuming a standard deviation of 15. The PDI, MDI and fluency score had a normal distribution. The distribution of the NOS was skewed to the left. In order to achieve normality, we performed the following transformation: -log (58.5-NOS). The analysis focused on the effect of type of feeding on the clinical neurological classification, the neurological optimality score, the fluency score and the Bayley PDI and MDI. Student's t-test was used to detect differences between the feeding groups in the transformed NOS, the fluency score, and the PDI and MDI. The χ^2 test was used to detect differences in the presence of MND in the feeding groups. Linear regression analysis was applied to adjust for potential confounders. For the calculation of the effect of the type of feeding, three dummy variables were created: one denoting the intake of CF, one indicating the consumption of LF and the third denoting breast milk intake. Because two comparisons were made between the feeding groups, the significance level was set to 0.025 (Bonferroni corrected).

Results

None of the children showed a definitely abnormal neurological condition such as cerebral palsy. In the CF group 8 (5%) of the children were classified as MND, in the LF group 10 (7%) and in the BF group 5 children (3%). The rate of MND did not differ significantly between the three groups. The median of the NOS in each feeding group was 52 and did not differ significantly between the three feeding groups (Table 2). Also, the mean of the fluency score did not differ significantly between the three groups. It was 9.8 in the CF group, 10.0 in the LF group and 10.0 in the BF group (Table 2).
The mean value of the Bayley PDI in the CF group was 100.9, in the LF group 99.4 and in the BF group 103.2. The mean MDI values were 105.4, 102.7 and 107.5, respectively (Table 2). The values did not differ significantly between the three groups.

TABLE 2. Developmental outcomes at the age of 18 months

	CF-group	LF-group	BF-group
Neurological Optimality Score, median (P5; P95)	52 (42; 55)	52 (42; 55)	52 (42; 56)
Number of children with MND	8 (5%)	10 (7%)	5 (3%)
Fluency cluster score (mean ± SD)	9.8 ± 2.0	10 ± 1.8	10 ± 1.9
Bayley PDI (mean ± SD)	100.9 ± 13.6	99.4 ± 13.4	103.2 ± 14.5
Bayley MDI (mean ± SD)	105.4 ± 15.0	102.7 ± 15.4	107.5 ± 16.0

Multivariate analysis confirmed that type of feeding did not explain the NOS, the fluency score, MDI or PDI (Table 3). Factors which were associated with a higher neurological optimality score were a higher HOME score, a higher age at assessment and a lack of participation in a parenthood course. A lower fluency score was related to the following items of the OOS: unreliable date of the last menstrual period, the need of uterus stimulation during labour and neonatal jaundice requiring medical intervention. Factors which explained the MDI were parity, maternal education and the HOME score. The PDI could be explained by paternal education and the HOME score. Developmental outcome was better when parental education was higher and the home environment was more affluent.

Table 3. Multivariate models explaining the MDI, PDI, NOS and fluency score

	Standardized Coefficients	Significance	Explained variance (%)[a]
Neurological examination by Hempel			
Neurological Optimality score (NOS)			14.6
Took part in a parenthood course	-0.11	0.01	
Age of infant at investigation (weeks)	0.35	<0.0005	
HOME-score	0.09	0.04	
Dummy variables (BF, CF, LF)	-	NS	
Fluency Cluster Score			13.5
Uncertain or unreliable date of LMP	-0.12	0.008	
Augmentation of labour	-0.10	0.02	
Neonatal jaundice requiring medical therapy	0.08	0.06	
Dummy variables (BF, CF, LF)	-	NS	
Bayley Scores of Infant Development			
Mental Development Index (MDI)			14.3
Parity	-0.20	<0.0005	
High education partner gravida	0.22	<0.0005	
HOME-score	0.21	<0.0005	
Dummy variables (BF, CF, LF)	-	NS	
Psychomotor Development Index (PDI)			5.1
High education partner gravida	0.19	<0.0005	
HOME-score	0.10	0.05	
Dummy variables (BF, CF, LF)	-	NS	

[a]Adjusted r^2

Discussion

Our study indicates that the beneficial neurodevelopmental effect of 2 mo LCPUFA supplementation in healthy term infants, which was present at 3 mo, can not be detected at the age of 18 mo. The fact that we were unable to detect the effect of LCPUFA supplementation cannot be attributed to the power of the study, as the *a priori* power analyses had indicated that the present group sizes and evaluation tools would allow for the detection of a difference of 5 points of the Bayley indices. A difference of 5 points, i.e. 0.33 SD, can be regarded as the minimum effect size which can be considered as clinically significant for health of the general population. It should be noted, however, that nutritional interventions that have resulted in public health changes have been associated with greater effect sizes as measured by the Bayley scales [18]. A possible explanation for the fact that we did not find a beneficial effect of LCPUFA supplementation at the age of 18 mo is that the subtle effects of LCPUFA supplementation are transient and do not affect neurodevelopmental condition beyond the age of 1 y. Such a transient effect of postnatal LCPUFA supplementation has been reported before for psychomotor development and visual function [8,19,20]. Up until now, only two studies have been able to find a positive effect of postnatal LCPUFA supplementation in term infants on the child's condition after

the age of 6 mo. Birch et al. found a positive effect of LCPUFA supplementation for a period of 17 wk on the Bayley MDI at 18 mo [21]. A similar effect on the PDI was absent. Willatts et al. found a positive effect of LCPUFA supplementation during the first 4 postnatal months on problem solving at the ages of 10 mo [22]. There are several arguments which support our conclusion that postnatal LCPUFA supplementation in term infants does not influence neurodevelopmental condition at 18 mo. Our finding that LCPUFA do not influence neurodevelopment at 18 mo of age is consistent with the outcome of five other randomized trials in which full-term infants received LCPUFA for at least 4 mo and the children were followed until the age of 12–24 mo [19,23–26]. These studies used general measures, such as the Bayley scales, to determine neurodevelopmental outcome. A recent study by Auestad et al., in which the effect of LCPUFA supplementation in term infants on IQ, vocabulary, and visual-motor function and visual acuity at the age of 39 mo was assessed, was also unable to demonstrate a positive effect of LCPUFA [27]. In the present study, we used the sensitive neurological assessment technique of Hempel, which is able to detect minor degrees of neurological dysfunction. For instance, the subtle effects of pre- and postnatal PCB exposure on neurodevelopment can be demonstrated with the Hempel technique [11]. Nevertheless, we were unable to demonstrate a beneficial effect of LCPUFA on neurological condition at 18 mo. The finding that postnatal LCPUFA supplementation does not affect subtle neurodevelopmental outcome at the age of 18 mo does not preclude a beneficial effect of postnatally acquired LCPUFA on neurodevelopmental outcome at a later age. The age of 18 mo is an age at which it is notoriously difficult to detect subtle signs of dysfunction. It is a period of a transient latency in the expression of minor neurological dysfunction [28]. Previously, we found that LCPUFA supplementation during the first 2 mo after birth in term infants induces a better quality of general movements at the age of 3 mo [6]. An abnormal quality of general movements is related to the development of minor neurological dysfunction (MND), aggressive behaviour and attention problems at school age [7]. Recent findings indicated that abnormal GMs are related in particular to dysfunctions in the domains of fine manipulative ability and co-ordination [29]. In other words, the abnormal GMs point specifically to dysfunctions in the (sub)cortical circuitries (the cortico–striato–thalamo–cortical and cerebellar– thalamo–cortical pathways). Interestingly, it is especially these parts of the brain which seem to be affected by LCPUFA supplementation [30]. Thus, it is conceivable that LCPUFA supplementation in term infants could induce a better condition of specific cognitive outcomes at school age, notwithstanding the presence of a period at toddler age where the advantage cannot be detected. The study of Helland et al. on LCPUFA supplementation of pregnant and lactating women suggests the effect of pre- and postnatal LCPUFA supplementation might be stronger than the effect of supplementation restricted to the postnatal period only [31]. The study of Helland et al. was able to demonstrate an effect of pre- and postnatal LCPUFA supplementation on global IQ scores at 4 y of age [31]. However, it is also possible that the latter positive effect was induced by

the high rate of attrition in the study, as only about a quarter of the group included in the study at birth was re-assessed at the age of 4 y. The comparison of the formula-fed groups and the breastfed group showed no differences in the neurological outcomes, even when confounding factors were taken into account. Lucas et al. also found no beneficial effect of breastfeeding given for at least 6 wk on the MDI and PDI at the age of 18 mo [24]. Similarly, Auestad et al. could not demonstrate a positive effect of exclusive breastfeeding for at least 3 mo on MDI and PDI at the ages of 6 and 12 mo [24]. Thus, our study strengthens the evidence that it is difficult to detect at the age of 18 mo potential beneficial effects of breastfeeding on neurological and cognitive development in healthy term infants. A positive effect of breastfeeding has been demonstrated on neurological condition at 3 mo [6]. Also, at older ages, when the nervous system has developed more complex neural functions, the positive effect of breastfeeding has been demonstrated in various studies [3,5]. The youngest age beyond the age of 24 mo at which the effect has been demonstrated is 42 mo [4]. This implies that, with respect to the detection of the positive effect of breastfeeding on neurodevelomenal outcome, the period between 12 and 24 mo can be regarded as a silent period. In summary, we found no significant effect of LCPUFA supplementation during the first 2 postnatal months or breast-feeding on sensitive markers of neurodevelomental outcome at the age of 18 mo. However, these findings do not necessarily rule out that there might be a beneficial effect of LCPUFA supplementation on specific forms of neurodevelopmental outcome at school age, as the age of 18 mo appears to be a difficult one at which to detect subtle neurodevelopmental differences between groups.

Acknowledgements

We kindly acknowledge the help of Dr J. A. L. Wildeman, Dr H. M. Tjoonk and Ms C. A. Veltman-Starkenburg in the collection of the data, and IrRR Bakker for various forms of technical assistance. The study was financially supported by Numico Research BV.

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Neonatal fatty acid status and neurological development

3

3.1

Relationship between umbilical cord essential fatty acid content and the quality of general movements of healthy term infants at 3 months

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Pediatric Research 2006;59:717-22

Abstract

Prenatal essential fatty acid (EFA) status might be an important factor in the development of the central nervous system (CNS). The aim of the present study was to evaluate the relationship between the fatty acid compositions of the umbilical blood vessels at birth, used as a proxy of prenatal EFA status, and quality of general movements (GMs) at 3 mo. Umbilical artery and vein fatty acid compositions were investigated in a mixed group of breastfed infants and infants fed with formula with or without long-chain polyunsaturated fatty acid (LCPUFA) supplementation. At the age of 3 mo, video assessment of the quality of GMs was performed to evaluate neurologic condition. The quality of GMs was scored by assessing the degree of variation, complexity, and fluency. Outcomes were classified as normal-optimal, normal suboptimal, mildly abnormal, and definitely abnormal movements. Information on potential confounders, including the type of postnatal feeding, was collected prospectively. Associations between fatty acid status at birth and quality of GMs were investigated, and multinomial logistic regression analyses were carried out. None of the infants showed definitely abnormal movements. Infants with mildly abnormal GMs had a lower EFA index, lower arachidonic acid (AA) content, higher total n-9 fatty acid, and higher total monounsaturated fatty acid (MUFA) content in the umbilical artery compared with infants with normal GMs. Multivariate analyses confirmed these findings. We conclude that mildly abnormal GMs are associated with a less favourable EFA status in the umbilical artery.

Introduction

LCPUFAs are abundantly present in the CNS of infants (1). Their accumulation in the brain occurs, especially during the last trimester of gestation and during early postnatal life (1,2), a process that is affected by nutrition (3,4). It is conceivable that dietary supplementation of LCPUFAs enhances incorporation of LCPUFAs into the membranes of the CNS and possibly improves the neurologic condition of the young child. At present, there is increasing evidence of a beneficial effect of LCPUFA supplementation on visual function and psychomotor development in preterm and term infants (5–7). No consistent evidence has been provided of a positive effect of LCPUFA supplementation on developmental outcome at 1 to 3 y, and no data are available on the effect of LCPUFA supplementation on neurobehavioral development beyond the age of 3 y (8–10). Less is known about the effect of the infant's neonatal fatty acid status on neurodevelopmental outcome. A recent study by our own group indicated that a lower neonatal status of docosahexaenoic acid [(DHA), a member of the n-3 fatty acid series], AA (a member of the n-6 fatty acids), EFA in the umbilical vein is associated with a less favorable neurologic condition on postnatal days 10 to 14 (11). Helland *et al.* (12) reported that supplementation with cod liver oil (rich in n-3 fatty acids) during pregnancy and lactation resulted in higher levels of n-3 fatty acids in blood plasma at birth, which were associated with more mature electroencephalography (EEG) scores on the second day of life. Neonatal fatty acid status was not related to EEG maturity at 3 mo. Follow-up at 4 y indicated that neonatal DHA in plasma at birth was not related to intelligence (13). A negative correlation was found between neonatal Mead (20:3n-9) and Osbond (22:5n-6) acids, which represent lower EFA status and intelligence at 4 y (13). Malcolm *et al.* (14), in a study in which pregnant women received supplementation with either fish oil capsules rich in DHA or placebo capsules, did not find a relationship between the infant's DHA status in red blood cells and plasma at birth and latencies to visually evoked potentials recorded to flash stimuli shortly after birth and at the ages of 10 wk and 6 mo. However, they did find a relationship between neonatal DHA status and latencies to visually evoked potentials recorded to pattern-reversal stimuli at 10 wk and 6 mo. Two other studies found no relationship between neonatal LCPUFA status and cognitive function at 4 and 7 y of age (15,16). Taken together, it is as yet unclear whether neonatal LCPUFA status affects neurodevelopmental outcome, and if so, which LCPUFA plays a prominent role. To detect the possible subtle effects of neonatal fatty acid status on neurologic condition, sensitive evaluation instruments are required. Previous studies by our group indicated that the assessment of the quality of GMs is a sensitive tool for evaluating the effect of nutritional status on neurologic condition (6,17). GMs are spontaneous movements involving all parts of the body. They arise early in fetal life and persist until 3–4 mo after term age when goal-directed behavior emerges. Normal GMs are characterized by variation, complexity, and fluency. These characteristics disappear when movements become abnormal. The presence of definitely abnormal GMs at 2–4 mo

predicts the development of cerebral palsy with a high accuracy (18 –20). The presence of mildly abnormal GMs at 2–4 mo is associated with an increase in the risk of the development of minor neurologic dysfunction, attention problems, and aggressive behavior at school age (19 –21). In previous studies, we evaluated the effect of LCPUFA supplementation of infant formula and that of the influence of duration of breastfeeding on the quality of GMs (6,17). None of the participating healthy term infants had definitely abnormal GMs. We found that LCPUFA supplementation was associated with a reduction of mildly abnormal GMs and that breastfed infants showed better movement quality when they had received human milk for more than 6 wk. The present paper is based on the groups of infants who participated in the LCPUFA supplementation study. We addressed the question whether the fatty acid composition of the umbilical blood vessels at birth of infants who showed mildly abnormal GMs at 3 mo differed from that of infants with normal GMs. The problem was tackled with multivariate statistics to be able to take into account the role of type of postnatal feeding and other confounders.

Subjects and methods

Subjects

This study is part of a double-blind, randomized, controlled trial investigating the effect of LCPUFA supplementation on neurodevelopment of healthy term infants. The study has been described in detail in Bouwstra *et al.* (6). Mother-infant pairs were recruited during pregnancy check-up visits at various locations in and near Groningen. Final enrollment in the study occurred in the neonatal period, at which time parents provided written informed consent. All infants were born at 37–42 wk of gestation, had a native West European origin, and were born between February 1997 and October 1999. Excluded from the study were children with a congenital disorder interfering with adequate functioning in daily life, children from multiple births, children whose mother did not master the Dutch language or had significant illness or disability, and adopted and fostered children. The study population consisted of 474 infants. There were three study groups: two randomized formula groups and one group of mothers who decided to breastfeed their infants (n = 159). The control formula group (n = 169) received commercial formula feeding (Nutrilon Premium; Nutricia, Zoetermeer, the Netherlands) for 6 mo and the LCPUFA-supplemented formula group (n = 146) received the same formula enriched with 0.45% AA and 0.30% DHA for 2 mo. After 2 mo, the LCPUFA-supplemented group received control formula feeding until the age of 6 mo. Breastfeeding was given as long as possible. If breastfed infants required formula feeding as a supplement or when breastfeeding stopped, they received LCPUFA supplemented formula until 2 mo and control formula from 2 to 6 mo. Parents and examiners were unaware of the type of formula feeding that the infants received. The study

was approved by the Ethics Committee of the University Medical Centre Groningen (MEC 95/08/207).

Analysis of fatty acids of the umbilical vessels

Permission to obtain umbilical cord tissue was granted in 317 infants, *i.e.* in 67% of the original population (Fig. 1). The umbilical cord was immediately collected after parturition. Details on the collection and processing can be found in Muskiet *et al.* (22). Seven to 10-cm samples were taken at the most proximal site of the placenta and stored in saline at 4°C for a maximum duration of 24 h until further processing for fatty acid analyses according to established methods (22). Fatty acid methyl esters, *trans*-fatty acids included, were determined by high-resolution capillary gas liquid chromatography as described by Dijck-Brouwer *et al.* (11) and Decsi *et al.* (23). Data were expressed as percentages of weight of fatty acids with chain lengths of 14–24 carbon atoms. EFA status was derived from 20:3n-9 (Mead acid), 20:3n-9/20:4n-6, and the EFA index
(n-3 + n-6)/(n-7 + n-9), while the DHA deficiency index (22:5n-6/22:4n-6) was used to describe DHA status (23).

Neurologic assessment at 3 months

Follow-up at the age of 3 mo was performed for 269 of the infants for whom information on fatty acid status in the umbilical cord was available (Fig. 1). The major reason that infants were not assessed at 3 mo was an overload of work for the research team. The social and pre- and perinatal backgrounds of infants who were included in the present analyses are shown in Table 1. Social and pre- and perinatal backgrounds of infants included in the present analyses were in general similar to those of the infants who were not included into the analyses because of missing fatty acid samples or missing GM assessment. However, infants not included in the study did have a slightly lower obstetric optimality score (OOS) (mean values: 62 *versus* 61, $p < 0.05$) and more had fathers who smoked (51% *versus* 41%, $p < 0.05$). For more details, see section on assessment of potential confounders. The neurologic assessment consisted of the evaluation of the quality of GMs. The infants' spontaneous motility was videotaped for 15 min in the home environment. During the recording, the infant was dressed in his or her underwear, and he or she was lying in supine position. Care was taken to only record GMs when the infants were awake, active, and not crying. Investigators who were blinded to the subjects' group assignments analyzed the quality of the videotaped GMs. Movements were classified as normal-optimal, normal-suboptimal, and mildly abnormal. The classification in categories was based on the criteria shown in Table 2. Experienced observers evaluated these criteria, which are the three main characteristics of GMs: (1) GM complexity, denoting the spatial variation of the movements; (2) GM variation, which represents the temporal variation of the movements; (3) GM fluency, which indicates the presence of smooth, subtle, and graceful movements. The interscorer agreement on GM quality is good: $\kappa = 0.81$ (24). As mentioned before,

none of the infants had definitely abnormal GMs. For the present analyses, the infants with normal-optimal and normal-suboptimal GMs were pooled into one group.

TABLE 1. Social and perinatal characteristics of the infants included in the analyses

Variables	Total group	Normal GMs	Mildly abnormal GMs	P-value*
Number of children	269	205	64	
Male Gender (%)	54 %	53 %	59 %	NS
Birthweight, mean ± SD (g)	3551 ± 424	3571 ± 419	3488 ± 419	NS
Birth order: first born (%)	43 %	41 %	48 %	NS
Maternal age (years), mean ± SD	30 ± 4.3	31 ± 4.2	30 ± 4.6	NS
Maternal education: higher education (%)	22 %	23 %	19 %	NS
Paternal education: higher education (%)	23 %	25 %	18 %	NS
Maternal smoking during pregnancy (%)	25 %	25 %	24 %	NS
Paternal smoking during pregnancy (%)	41 %	41 %	39 %	NS
Maternal alcohol consumption during pregnancy (%)	14 %	12 %	22 %	0.04
Obstetrical Optimality Score†, mean ± SD	62 ± 3.7	62 ± 3.6	62 ± 4.1	NS
HOME score ‡, mean ± SD	43 ± 2.1	43 ± 2.1	43 ± 2.2	NS

* Difference between infants with normal GMs and those with mildly abnormal GMs.
† The obstetric optimality score consists of 74 variables describing the obstetric condition, ranging from the parents' socioeconomic status and health condition to the infant's condition immediately after birth (31)
‡ Home Observation for Measurement of the Environment (HOME) inventory (32).
NS, not significant.

TABLE 2. Classification of the quality of GMs

Classification	Complexity[2]	Variation[3]	Fluency[4]
Normal-optimal	+++	+++	+
Normal-suboptimal	++	++	—
Mildly abnormal	+	+	—
Definitely abnormal	—	—	—

[1] From reference 11. +++, abundantly present; ++, sufficiently present; +, present, but insufficiently; -, absent.
[2] Defined as spatial variation. The infant actively produces frequent changes in the direction of movement of the participating body parts. The changes in direction are brought about by continuously varying combinations of flexion-extension, abduction-adduction, and endorotation-exorotation of the participating joints.
[3] Defined as temporal variation. Across time, the infant produces continuously new patterns of movement, ie, the infant has an apparently infinite movement repertoire.
[4] Defined as the presence of smooth, supple, and graceful movements. Fluency in particular points to the velocity profile of the movements, which is characterized by gradual accelerations and decelerations.

Assessment of potential confounders

At enrollment, detailed and standardized information was collected on the infants' social and pre- and perinatal conditions. For the latter, we used the 74 variables of the OOS, ranging from the parents' socioeconomic status and health condition to the infant's condition immediately after birth. The sum of all items having a value within a predefined optimal range forms the OOS for an infant (25). We used the information obtained from the OOS both at the single-item level where the items were dichotomized into optimal and nonoptimal categories and in the form of the sum of all items of the OOS. Besides collecting information on the parents' level of education and occupation, social condition was also assessed by the HOME inventory (26). Information on the distribution of confounding variables in the groups of infants with normal and mildly abnormal GMs are presented in Table 1.

Statistics

The analyses focused on the differences in fractions of fatty acid composition of umbilical vessel wall between infants with normal GMs and those with mildly abnormal GMs. First, univariate statistical analyses were performed with the Mann-Whitney test to calculate differences in fatty acid composition in umbilical vein and artery wall between children with normal and mildly abnormal GMs. Next multivariate analyses were carried out by means of multinomial logistic regression analyses. This allowed us to elucidate the relationship between fatty acid status at birth and GM quality while correcting for type of postnatal feeding and potential confounders such as the postnatal age of the infant at GM assessment, paternal smoking, and the total OOS. For calculating the effect of postnatal feeding, a dummy variable was created for the four following nutritional groups: LCPUFA formula, control formula, breastfeeding for $\leq$ 6 wk, breastfeeding > 6 wk. Nonparametric statistics were used as the large majority of variables were not normally distributed. *p*

Values < 0.05 were considered significant. Statistical analyses were performed using SPSS 10 (SPSS Inc., Chicago, IL).

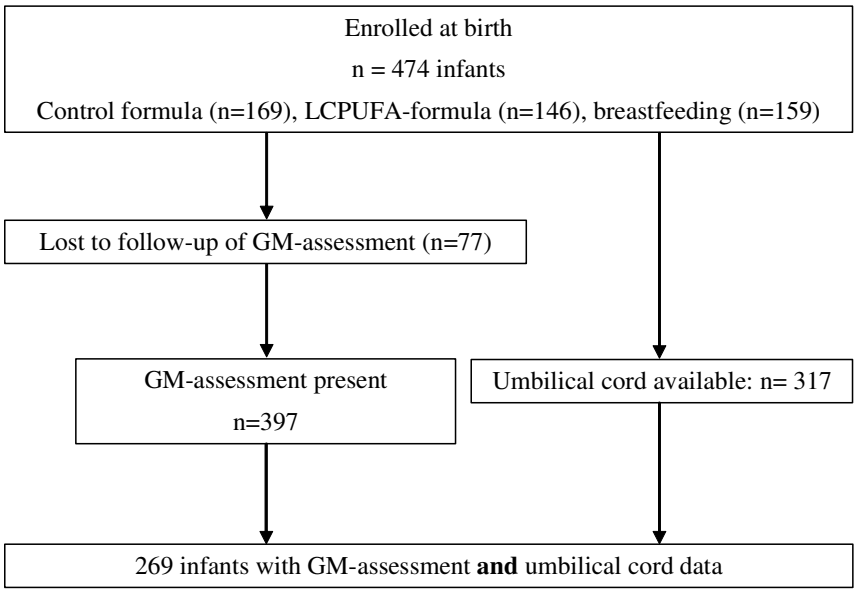

FIGURE 1. Flow diagram of infants included into the present study.

Results

The fatty acid composition of the umbilical veins of infants with mildly abnormal GMs did not differ significantly from that of infants with normal GMs (Fig. 2). However, the fatty acid composition of the umbilical artery did differ between the infants with mildly abnormal GMs and those with normal movements (Table 3). Univariate analyses indicated that the umbilical artery of infants who showed mildly abnormal GMs contained more MUFAs and n-9 fatty acids (especially 18:1n-9, *i.e.* oleic acid) and contained fewer LCPUFAs, in particular less AA, a lower EFA index, and a higher DHA deficiency index than the artery of infants with normal GMs (Fig. 2). *Trans*-fatty acid content of the umbilical artery of infants with mildly abnormal GMs did not differ from that of infants with normal GMs. Multivariate analyses confirmed all but one of these relationships: the relationship between DHA deficiency index and GM quality disappeared when correcting for confounders (Table 4). Confounders that contributed consistently and significantly to the risk of showing mildly abnormal GMs were a lower age at GM assessment, the mother not being married during pregnancy, and the condition of the perineum, *i.e.* the presence of

either an intact perineum or a total rupture. Having been breastfed for > 6 wk was consistently associated with a lower risk of mildly abnormal GMs. We assume that the association between the condition of the perineum and the quality of GMs was a chance finding.

TABLE 3. Fatty acid composition of umbilical artery wall of infants with GM-assessment

Fatty acid, umbilical artery	Infants with normal GMs (n = 205)		Infants with mildly abnormal GMs (n = 64)		Univariate analyses p value	Multivariate Analyses,* P value
	Median %	Range	Median %	Range		
Sum SAFA	49	46-59	49	44-53	NS	NS
Sum MUFA	20	14-26	21	17-25	0.003	0.003
Sum n-9 fatty acids	19	11-26	20	15-25	0.002	0.001
18:n-9	11	8-16	12	8-16	0.006	0.006
Sum LCPUFA	29	22-34	28	22-33	0.04	0.03
20:3n-6	2	1-3	1	0.8-2	0.03	0.02
20:4n-6	13	9-19	12	9-16	0.04	0.03
22:4n-6	3	2-6	3	2-4	NS	0.02
22:5n-6	4	2-6	4	2-5	NS	NS
22:6n-3	5	1-7	4	3-7	NS	NS
Total Σ n-6 and n-3	27	19-33	26	19-30	0.03	0.02
Total *trans*-fatty acids	0.7	0.4-3	0.6	0.4-2	NS	NS
DHA deficiency index	1	0.4-3	1	0.6-2	0.04	NS
20:3n-9 / 20:4n-6	0.2	0-0.6	0.2	0.1-0.5	0.04	0.048
EFA index	1	0.7-3	1	0.7-2	0.01	0.003

Values are %wt.
* See Table 4.
SAFA, saturated fatty acids; DHA deficiency index, 22:5n-6/22:4n-6; EFA index, (n-3 + n-6)/(n-7 + n-9). NS, not significant.

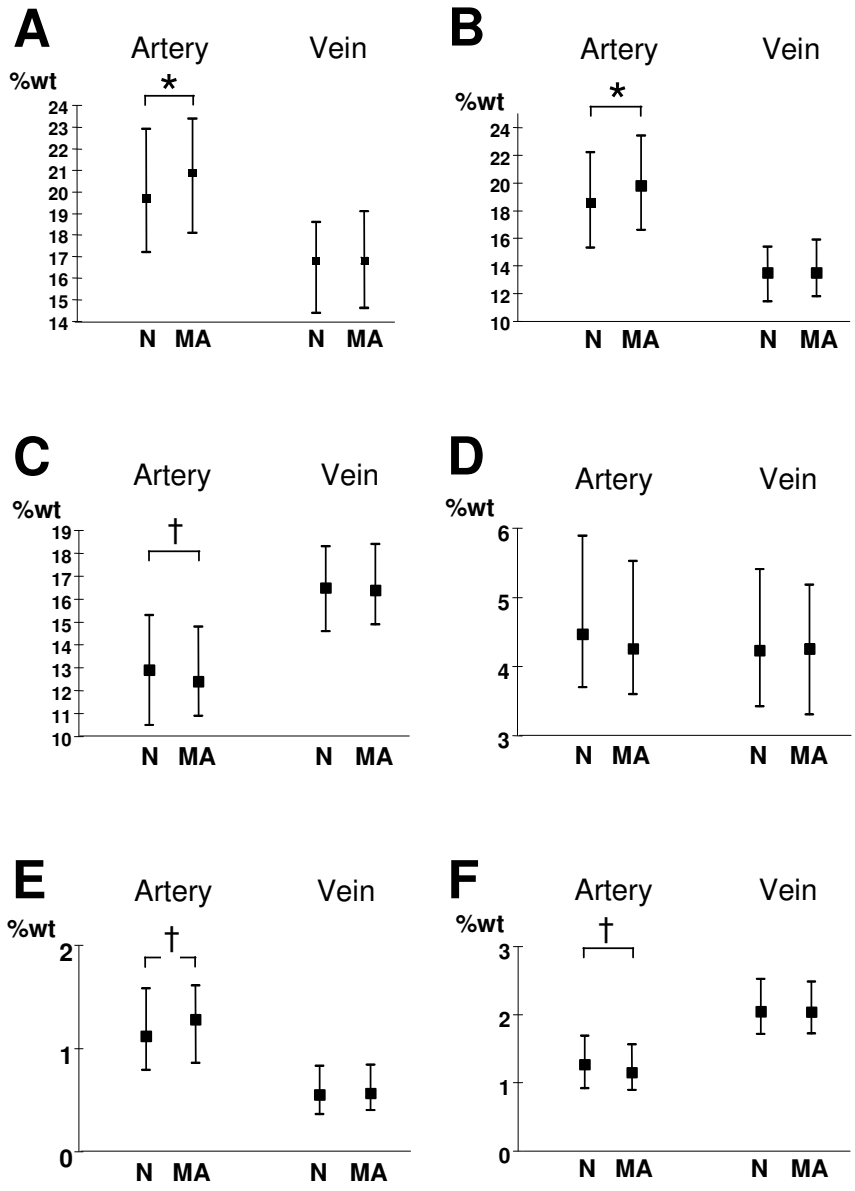

FIGURE 2. Differences in the fatty acid composition of umbilical cords between infants with normal (N) and mildly abnormal (MA) GMs. (*A*) Total MUFA, (*B*) total of n-9, (*C*) AA, (*D*) DHA, (*E*) DHA -eficiency index, (*F*) EFA index. The vertical lines represent the ranges between the 10th and 90th percentiles of values of the percentage of weight; the horizontal bars show the median values. *$p < 0.01$; †$p < 0.05$.

Table 4. Results of logistic regression analyses of the contribution of fatty acid status at birth to the occurrence of mildly abnormal GMs, corrected for potential confounders such as postnatal feeding group

Factors	**Standardized coefficients*** **(95% CI)**	**p**	**Explained variance (%)** Cox and Snell
Umbilical artery			
Sum MUFA	1.27 (1.09, 1.40)	0.003	17%
Sum n-9 FA	1.23 (1.09, 1.39)	0.001	24%
18:1n-9	1.33 (1.10, 1.60)	0.006	23%
Sum LCPUFA	0.86 (0.74, 0.99)	0.03	13%
20:3n-6	0.33 (0.13, 0.85)	0.02	14%
20:4n-6	0.81 (0.68, 0.98)	0.03	13%
22:4n-6	0.50 (0.27, 0.90)	0.02	19%
22:5n-6	1.0 (0.62, 1.8)	N.S.	17%
22:6n-3	0.74 (0.48, 1.1)	N.S	18%
Total n-3 and n-6	0.87 (0.78, 0.97)	0.02	14%
Total *trans*-fatty acids	1.8 (0.37, 8.9)	N.S.	12%
DHA deficiency index†	2.0 (0.73, 5.2)	N.S.	12%
20:3n-9 / 20:4n-6	20 (1.01, 397)	0.048	13%
EFA index	0.16 (0.045, 0.53)	0.003	15%

* A standardized coefficient of > 1 means higher risk of the occurrence of mildly abnormal GMs; a standardized coefficient < 1 means a lower risk of the occurrence of mildly abnormal GMs.
† Only significant in the univariate analysis.

Discussion

Our study indicates that mildly abnormal GMs at 3 mo are associated with a lower AA and EFA status of the umbilical artery at birth. The neonatal DHA status was not related to neurologic condition as measured by GM quality, *i.e.* the association between DHA deficiency index and GM quality was present in the univariate analysis, but disappeared in the multivariate analysis. In addition, we found that mildly abnormal GMs were associated with a higher level of monounsaturated fatty acids and with a higher total concentration of n-9 fatty acids, in particular, of oleic acid, in the umbilical artery at birth. The associations between fatty acid compositions of the umbilical artery remained statistically significant when taking into account important confounders such as type of postnatal feeding, age at investigation, and obstetric and social factors including smoking and maternal alcohol consumption during pregnancy. Univariate analysis indicated that maternal alcohol use during pregnancy was related to the occurrence of mildly abnormal GMs. This association disappeared in the multivariate analysis. No indications were found that maternal alcohol consumption modified the relationships between neonatal fatty acid status and the quality of GMs. It is well-known that prenatal alcohol exposure may affect the condition of the fetal brain (27). However, our data indicated that the effect of prenatal fetal fatty acid status

on postnatal neurologic condition is stronger than that of modest prenatal alcohol exposure. Interestingly, the randomized allocation into the standard formula group or LCPUFA-supplemented formula group during the first 2 mo after birth no longer had an effect on the quality of GMs when neonatal fatty acid status was taken into account (6). This suggests that neurologic condition rather depends on the prenatal essential fatty acid status than on the type of postnatal feeding. The differences in fatty acid compositions of the umbilical artery between children with normal and mildly abnormal GMs were relatively small. Nevertheless, the standardized coefficients in the multinomial regression analysis indicated that even small differences in fatty acid composition significantly affect neurologic condition at 3 mo and thus might be considered clinically relevant. It is interesting to note that we found relationships between GM quality and fatty acid composition in the umbilical artery and not in the umbilical vein. The umbilical vein represents the supply of fatty acids from the placenta and the fat stores of the mother. The umbilical artery fatty acid composition represents the downstream of fatty acids from the fetus that is positively affected by placental supply and fetal synthesis of fatty acids and negatively influenced by fetal extraction. We did not find any differences in umbilical vein fatty acid composition between infants with normal GMs and mildly abnormal GMs. This might imply that an inadequate maternal fatty acid supply was not a determinant of the quality of GMs in this study population. Instead our data suggest that the development of mildly abnormal GMs might be associated particularly with the impaired extraction or increased dilution of AA and EFA driven by fetal metabolism and not by maternal supply. It could be that dilution of EFA and LCPUFAs in the umbilical artery is the result of an increased maternal supply of glucose to the placenta that induces extra *de novo* fatty acid synthesis from glucose, especially saturated fatty acids and MUFAs in the fetus (11,28). This suggestion fits with our finding that the arteries of infants with mildly abnormal GMs contained relatively more MUFAs. In general, DHA is considered to be the most important LCPUFA for early brain development (29). However, the evidence of this idea basically stems from supplementation studies carried out during postnatal life (30). In the present study, we found that neonatal AA status, in particular, was related to neurologic outcome. The relationship between the DHA deficiency index of the umbilical artery and GM quality disappeared when the association was corrected for confounders. This could imply that during neonatal life, the supply of AA to the fetus is more critical than the supply of DHA. This hypothesis is supported by the finding that during gestation the accretion rates of n-6 fatty acids in the fetal brain are approximately twice as high as those of n-3 fatty acids (31). After birth, accretion of DHA seems to become more critical because after term age, brain DHA content increases with increasing age, whereas brain AA content remains quite constant (1). Another finding that supports the idea that accretion of AA is more critical than DHA accretion is the lower AA content in the umbilical artery compared with the umbilical vein (median 12.8% and 16.5%, respectively), whereas less difference existed between DHA content of the umbilical vein and umbilical artery (median 4.4% and 4.3%, respectively).

Also the study of Agostoni *et al.* (32), which found a significant relationship between early postnatal AA status and psychomotor development at 5 mo in children with phenylketonuria underscores the importance of AA status in early life. Current data, including the data from this study, indicate that neonatal fatty acid status does not only affect neurologic status at birth (11,12) and maturation of latencies to visually evoked potentials at 10 wk and 6 mo (14), but also the neurologic condition at 3 mo. The finding that neonatal fatty acid status, in particular EFA and AA status, affects the prevalence of mildly abnormal GMs might be a finding with clinical relevance because it is known that the presence of mildly abnormal GMs is associated with an increased risk of the development of minor neurologic dysfunction and attention problems at school age (19 – 21). Until now, however, data on the long-term effects of fatty acid status of the neonate are inconclusive. Three studies have been carried out that were all characterized by high rates of attrition (13,15,16). The two studies, which were based on an observational design, were unable to find a relationship between maternal fatty acid status and cognitive function at school age (15,16). The intervention study of Helland *et al.* (13) was able to demonstrate a relationship between maternal DHA supplementation and cognition at 4 y. In conclusion, neonatal fatty acid status, in particular lower AA composition and EFA index in the umbilical artery, is associated with a less favorable neurologic condition at 3 mo of age as reflected by the presence of mildly abnormal GMs. Whether neonatal fatty acid status affects long-term neurodevelopmental outcome remains an open question. To answer this question, we need prospective observational and intervention studies with long-term follow-up and little attrition.

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3.2

Neurological condition of healthy term infants at 18 months: positive association with venous umbilical DHA status and negative association with umbilical *trans*-fatty acids

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Pediatric Research 2006;60:334-339

Abstract

Prenatal long-chain polyunsaturated fatty acids (LCPUFAs) and *trans*-fatty acids may affect neurodevelopment. In healthy term children, we determined relationships between relative fatty acid contents of umbilical arteries and veins and neurodevelopment at 18 months. The study comprised a mixed group of 317 breast-fed, formula-fed, and LCPUFA formula–fed children. Study endpoints were the Hempel neurologic examination resulting in a neurologic classification and neurologic optimality score (NOS), and the Bayley Psychomotor Developmental Index (PDI) and Mental Developmental Index (MDI). Fifteen children showed minor neurologic dysfunction (MND). The umbilical vein *trans, trans*-18:2n-6 content was higher in children with MND than in the normal group. The NOS was significantly reduced in infants with an umbilical vein docosahexaenoic acid (DHA) content within the lowest quartile. Umbilical vein arachidonic acid (AA) was related to NOS in univariate statistics but not in multivariate analyses. The sum of *trans*-fatty acids and that of C18 *trans*-fatty acids showed a negative association with NOS in both univariate and multivariate analyses. No associations were found between AA, DHA and total *trans*-fatty acids with PDI or MDI. In conclusion, neonates with a relatively low DHA status and those with high *trans*-fatty acid levels have a less favorable neurologic condition at 18 months.

Introduction

Little is known about the effects of prenatal essential fatty acid and long-chain polyunsaturated fatty acid (LCPUFA) status on long-term neurodevelopmental outcome in healthy term infants. This is remarkable because there are many indications that LCPUFAs play an important role in the development and function of the nervous system (1,2). LCPUFAs, notably DHA, affect biochemical properties of cell membranes and may alter signal transduction, gene expression, and cell function in the nervous system (1,3,4). Rapid accretion of LCPUFAs takes place in the infant's nervous tissue during pre- and early postnatal life when infants do not seem to synthesize sufficient amounts of LCPUFAs from their precursors to cover their high demands (5,6). The exclusive source of LCPUFAs during the prenatal period is supplied from maternal stores and maternal diet, a supply that is reflected by the LCPUFA content of the umbilical vein (7). Two recent studies indicated that the umbilical cord content of DHA, AA, and EFA is related to neurologic condition at postnatal day 10–14 and at 3 mo (8,9). Two other studies found no relationship between neonatal fatty acid status and cognitive function at 4 and 7 y of age (10,11). Koletzko (12) found that neonatal *trans*-fatty acid status is inversely correlated to birth weight in preterm infants. Little is known about the effects of *trans*-fatty acids on development and child health, but the available data indicate that *trans*-fatty acids may have adverse effects because of their negative association with LCPUFA status (13–15). A review by Larqué *et al.* (16) indicated that *trans*-fatty acid intake in adults averages 2–8 g/d (2.5% of total energy intake). This intake has been quite stable in the past decades due to a counterbalancing effect of more intake of hydrogenated oil and decreases in *trans*-fatty acid content in food (16). A report of the Dutch Ministry of Health, Welfare, and Sports indicated that adult intake of *trans*-fatty acids in The Netherlands in 2003 averaged 2.8 g/d (1.1% of total energy intake) (17). It is possible that *trans*-fatty acid exposure during early life may affect the infant's neurologic condition (16). The primary aim of this study is to evaluate in healthy term infants, the relationship between the relative LCPUFA content in the umbilical wall, used as a proxy of prenatal LCPUFA status, and neurodevelopmental outcome at 18 mo of age. The secondary aim was to study the relationship between *trans*-fatty acid content in the umbilical wall and neurodevelopmental outcome at 18 mo.

Methods

Subjects

This study is part of a double-blind, randomized, controlled trial investigating the effect of LCPUFA supplementation on neurodevelopment of healthy term infants. Details of the study design have been described elsewhere (18). Briefly, mother-infant pairs were recruited during pregnancy checkup visits at various locations in and near Groningen at

which time the parents provided written informed consent. Final enrollment in the study occurred in the neonatal period, at which time parents provided written informed consent. Enrollment occurred between February 1997 and October 1999. All infants were born at 37–42 wk of gestation and were of native West European origin. Excluded from the study were children with a congenital disorder interfering with adequate functioning in daily life, children from multiple births, children whose mother did not master the Dutch language or suffered from significant illness or disability, and children who were adopted and fostered. The study population comprised 474 infants. Three study groups were formed; two randomized formula groups and a breast-fed group (n=159). The control formula group (n=169) received a commercially available formula (Nutrilon Premium; Nutricia, Zoetermeer, The Netherlands) for 6 mo. The LCPUFA-supplemented formula group (n=146) received the same formula enriched with 0.45% AA and 0.30% DHA for 2 mo. After 2 mo, the LCPUFA-supplemented group received control formula until the age of 6 mo. Breast-feeding was done as long as possible. If breast-fed infants required formula feeding as a supplement or when breast-feeding stopped, they received LCPUFA-supplemented formula until 2 mo and control formula from 2 to 6 mo. The parents and the examiners were unaware of the type of formula that the infants received. The study was approved by the Ethics Committee of the Groningen University Hospital (MEC 95/08/207).

Analysis of fatty acids of the umbilical vessels

Permission to collect umbilical cord tissue was granted for 317 infants, *i.e.* 67% of the original population (Fig. 1). The umbilical cord was immediately collected after parturition. Seven- to 10-cm samples were taken at the most proximal site of the placenta and stored in saline at 4°C according to established methods (19). Fatty acid methyl esters were determined by high-resolution capillary gas liquid chromatography as described by Dijck-Brouwer *et al.* (8) and Decsi *et al.* (20). Data were expressed as % by wt of fatty acids with chain lengths of 14 to 24 carbon atoms (19). The detection limit of *trans*-fatty acids was one molecule among 10,000, *i.e.* 0.01%. A typical chromatogram of *trans*-fatty acids detected in our laboratory has been published (21)

TABLE 1. Social and perinatal characteristics of the infants

	Study group	No samples or no NOS available	p
Number of infants (%)	290 (61)	184 (39)	
Male gender (%)	55	52	NS
Birthweigth in g (mean ± SD)	3547 ± 430	3533 ± 501	NS
First born (%)	60	40	NS
Maternal age (years), mean ± SD	30 ± 4.2	30 ± 4.6	NS
Maternal higher education* (%)	22	20	NS
Paternal higher education* (%)	22	26	NS
Maternal smoking during pregnancy (%)	25	36	NS
Paternal smoking during pregnancy (%)	41	53	0.02
Maternal alcohol consumption during pregnancy (%)	13	17	NS
Obstetrical Optimality Score (mean ± SD)	62 ± 3.7	61 ± 4.7	0.05

* University education or vocational college.
NS, not significant.

Neurologic assessments at 18 months

Follow-up at the age of 18 mo consisted of two different neurodevelopmental assessments: the neurologic examination according to Hempel and the Bayley Scales of Infant Development (BSID). Follow-up for the various developmental outcome parameters in infants for whom information on fatty acid values in the umbilical cord was available is shown Figure 1. The social and perinatal background of the infants who were included in the present analyses, and the infants without assessments or fatty acid samples are shown in Table 1. Each child was assessed neurologically using the technique described by Hempel (22). It measures in a standardized free-field situation motor functions (grasping, sitting, crawling, standing, and walking). In addition to the assessment of motor milestones, the quality of motor behavior is also assessed. In addition, muscle tone, reflexes and the function of the cranial nerves are assessed. Each toddler was classified as neurologically normal, showing signs of minor neurologic dysfunction (MND), or as definitely abnormal. The classification of definitely abnormal implies the presence of a distinct neurologic syndrome, which leads to severe limitations in function and social participation, such as cerebral palsy. MND implies the presence of a functional impairment that may be associated with some degree of disability. Examples are mild deviations in gross and fine motor function or mild abnormalities in muscle tone regulation or reflexes. After classification into distinct categories, we used the optimality concept to summarize the neurologic condition. The optimal range was defined from 57 items of the neurologic examination list. The NOS is the sum of the number of items with outcomes within the predefined optimal range (23,24). The BSID-II was used to assess mental and psychomotor

development at the age of 18 mo (25). The Mental Developmental Index (MDI) and the psychomotor Developmental Index (PDI) were scored based on the number of items that were successfully completed. Because the children were not exactly 18 mo of age at the time of the assessment, we converted the scores into age-normalized values, as derived from recently developed Dutch norms (26). The MDI assesses memory, problem solving, discrimination, classification, language, and social skills. The PDI measures control of gross and fine muscle groups, including walking, running, jumping, grasping, and imitation of hand movements.

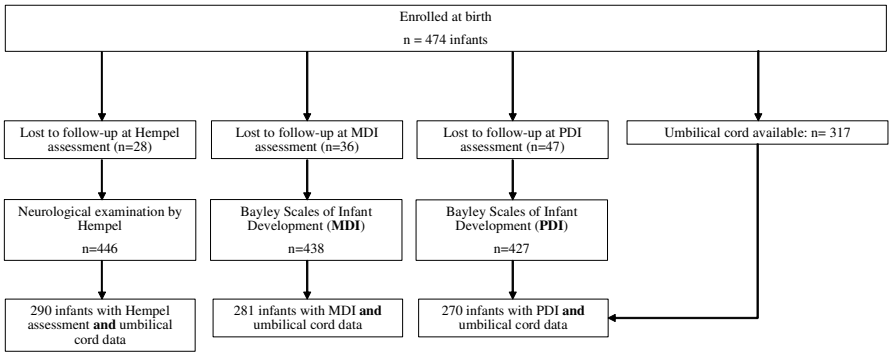

FIGURE 1. Flow diagram of infants included into the study and assessed at 18 mo of age. Total number of participants at enrolment: n = 474 {control formula group (n 169), LCPUFA formula (n = 146), breastfeeding (n 159)}.

Assessment of potential confounders

Detailed and standardized information on the infants' social and perinatal conditions was collected. For the latter, we used the 74 variables of the obstetric optimality score (OOS), which range from the parents' socioeconomic status and health condition to the infant's condition immediately after birth. The sum of the number of items having outcomes within a predefined optimal range forms the OOS score (27). We used the information obtained from the OOS both as raw data dichotomized into optimal and nonoptimal categories. Besides collecting information on the parent's level of education and occupation, social condition was also assessed by the Home Observation for Measurement of the Environment (HOME) inventory (28). The importance of taking into account the role of social factors in the analyses of the relationships between neonatal fatty acid status and developmental outcome is underlined by the fact that social economic characteristics and fatty acid status in the umbilical vein are related (Table 2) (17).

TABLE 2. Relationship between venous umbilical LCPUFAs, *trans*-fatty acids and social economic characteristics

	AA		DHA		Total c18 trans fatty acids		Total *trans*-fatty acids	
	Median	Range	Median	Range	Median	Range	Median	Range
Maternal education								
• Low	16.51	11.1-19.9	4.29	2.82-6.76	0.176	0.04-1.14	0.696	0.27-1.62
• High*	16.56	13.4-21.1	4.23	2.93-7.47	0.172	0.05-1.36	0.739	0.32-1.94
Paternal education								
• Low	16.63	11.1-21.1	4.28	2.82-6.76	0.165	0.04-1.14	0.686	0.31-1.62
• High*	16.28‡	13.1-19.8	4.26	3.07-7.47	0.200§	0.08-1.36	0.767§	0.27-1.94
Maternal profession								
• Low	16.53	11.1-21.1	4.24	2.82-6.76	0.171	0.04-1.32	0.687	0.27-1.92
• High†	16.48	13.4-19.8	4.40‡	3.14-7.47	0.177	0.05-1.36	0.739	0.33-1.94
Paternal profession								
• Low	16.61	11.1-19.8	4.27	2.82-6.76	0.168	0.04-1.14	0.697	0.31-1.62
• High†	16.31	13.4-21.1	4.27	3.07-7.47	0.191	0.05-1.36	0.714	0.27-1.94
Maternal smoking during pregnancy								
• No	16.43	11.1-21.1	4.25	2.82-7.47	0.175	0.04-1.36	0.704	0.27-1.94
• Yes	16.86‡	13.1-19.8	4.31	3.08-6.25	0.171	0.04-0.57	0.685	0.35-1.54
Paternal smoking during pregnancy								
• No	16.48	14.1-21.1	4.24	2.82-7.47	0.177	0.04-1.36	0.718	0.27-1.94
• Yes	16.54	11.7-19.9	4.27	2.76-6.25	0.161	0.04-0.57	0.685	0.33-1.54
Maternal alcohol use during pregnancy								
• No	16.54	11.1-21.1	4.24	2.82-6.76	0.170	0.04-1.36	0.691	0.27-1.94
• Yes	16.26	13.9-19.8	4.42	2.93-7.47	0.200‡	0.10-0.70	0.753	0.33-1.35

* University education or vocational college.
† Higher profession or independent middle class.
‡ P<0.05.
§ P<0.01.

Statistics

The PDI and MDI had a normal distribution. The distribution of the NOS was skewed to the left. To achieve normality, we performed the following transformation: -ln (58.5 - NOS). To calculate differences in fatty acid composition in umbilical vein and artery between the children with a normal neurologic condition and those with MND, we performed the Mann-Whitney test. The analyses focused on the relationship between the relative fatty acid contents of the umbilical wall (vein and artery) and the NOS and the Bayley MDI and PDI. Univariate nonparametric Spearman's correlations were used to calculate the correlations because most fatty acid data were nongaussian distributed. Subsequently, multivariate analyses were carried out by means of linear logistic regression analyses. This allowed elucidation of the relationship between the umbilical fatty acid content at birth and neurologic condition at 18 mo while correcting for potential confounders such as other umbilical fatty acids, type of postnatal feeding, the postnatal age of the infant at the time of follow-up assessment, paternal smoking, and the OOS. To calculate the effect of postnatal feeding, a dummy variable was created for the four

following nutritional groups: LCPUFA formula, control formula, breast milk for ≤ 6 wk, breast milk > 6 wk. p Values < 0.05 were considered significant. Statistical analyses were performed using the statistical package for social sciences (SPSS 10; SPSS Inc., Chicago, IL).

Results

Clinical neurologic classification

The umbilical fatty acids that were analyzed and related to neurodevelopmental outcome are presented in Table 3. None of the participants of the study showed a definitely abnormal neurologic condition such as cerebral palsy. Two hundred ninety children had a normal neurologic condition (95% of the study population) and 15 children had MND. The differences in fatty acid composition of the umbilical vein and artery between the neurologically normal children and those with MND are shown in Table 4. Children with MND had a significantly lower 20:0 content in their umbilical veins and arteries than neurologically normal children. Furthermore, *trans, trans*-18:2n-6 content in the umbilical vein was higher in children with MND than those in the normal group.

TABLE 3. Fatty acid composition (% by weight) of the umbilical wall of neurologically normal children and those with MND at 18 months

	Neurologically normal children (n=275)		**Children with MND (n=15)**		
	Median %	Range	Median %	Range	p
Vein					
20:0	0.43	0.09-2.65	0.36	0.18-0.44	0.006
Trans,trans-18:2n-6	0.07	0.00-0.25	0.10	0.03-0.16	0.04
Artery					
20:0	0.50	0.04-2.90	0.41	0.19-0.61	0.02

NOS

The correlation between the fatty acid composition of the umbilical vein and NOS is shown in Table 5. Univariate analysis revealed that AA in the umbilical vein showed a significantly positive relationship with NOS. The association was mainly explained by the difference in NOS between infants with umbilical vein AA content at or below the 50th percentile and those with umbilical vein AA content above the 50th percentile (p = 0.02, Fig. 2). The Spearman rank correlation did not reveal a relationship between DHA content of the umbilical vein and NOS (Table 5). However, closer inspection of the data indicated that infants with an umbilical vein DHA content within the lowest quartile had a significantly lower NOS than the other infants (p = 0.02, Fig. 2). Multivariate analysis could not confirm the positive association between raw AA or dichotomized AA data and

NOS. This approach did, however, confirm the association between the presence of umbilical vein DHA content in the lowest quartile and a lower NOS (p = 0.003, standardized β = 0.17). The sum of *trans*-fatty acids, the sum of C18 *trans*-fatty acids, and three individual *trans*-fatty acids of the umbilical vein showed significantly negative associations with NOS (Table 5, Fig. 2). The majority of these negative associations remained statistically significant in the multivariate analysis (Table 5). The negative association between the sum of *trans*-fatty acids and the NOS also remained statistically significant when we adjusted for DHA and AA content in the umbilical vein (p = 0.028, standardized β = -0.52) and when we repeated the analyses after exclusion of the breast-fed group (p = 0.007, rho = -0.20). The latter finding suggests that the association between *trans*-fatty acids and NOS may be attributed to prenatal and not postnatal *trans*-fatty acid exposure. No associations between essential fatty acids, LCPUFAs, or *trans*-fatty acids in the umbilical artery and the NOS were found.

Bayley Scales of Infant Development

Neither Univariate nor multivariate analyses revealed significant associations between AA, DHA, and total *trans*-fatty acids in the umbilical walls of artery or vein and the Bayley PDI and MDI (Fig. 2).

TABLE 4. Relative fatty acid composition of the umbilical vein and artery

	Vein				Artery			
Fatty acid	No.	Median % of weight	Range	% below detection limit	No.	Median % of weight	Range	% below detection limit
14:0	312	1.09	0.61–2.7	0	307	1.28	0.64–2.2	0
15:0	312	0.73	0.23–4.5	0	307	0.71	0.06–7.1	0
16:0	312	25.5	20.7–34.0	0	307	23.6	19.8–38.4	0
18:0	312	18.1	14.4–23.1	0	307	18.8	9.0–25.7	0
20:0	312	0.42	0.09–2.7	0	307	0.50	0.04–2.9	0
22:0	312	0.97	0.33–2.4	0	307	1.3	0.35–1.9	0
24:0	312	1.77	1.2–2.6	0	307	2.4	1.0–3.7	0
SAFAs	311	48.6	44.8–61.8		307	48.8	43.7–58.8	
18:3n-3	311	0.01	0.0–0.24	0	306	0.01	0.0–0.17	0
20:5n-3	311	0.01	0.0–0.14	6.8	306	0.02	0.0–0.17	4.9
22:5n-3	311	0.35	0.08–1.1	0	306	0.31	0.08–0.77	0
22:6n-3	311	4.3	2.8–7.5	0	306	4.4	1.4–7.1	0
Sum n-3	311	4.6	2.9–8.6		306	4.8	1.7–7.9	
18:2n-6	311	2.6	1.4–4.0	0	306	1.6	0.70–2.9	0
18:3n-6	311	0.044	0.0–0.38	0	306	0.07	0.0–0.74	0
20:2n-6	311	0.35	0.04–0.94	0	306	0.14	0.01–0.87	0
20:3n-6	311	2.2	0.89–3.8	0	306	1.5	0.73–2.8	0
20:4n-6	311	16.5	11.1–21.1	0	306	12.8	9.0–18.8	0
22:4n-6	311	5.2	2.3–8.5	0	306	3.0	1.5–5.6	0
22:5n-6	311	2.9	1.4–4.7	0	306	3.5	1.8–5.5	0
Sum n-6	311	30.1	19.0–35.0		306	23.3	15.7–31.4	
16:1n-7	311	0.53	0.22–1.1	0	306	0.52	0.07–1.1	0
18:1n-7	311	2.4	1.4–3.9	0	306	2.8	1.7–3.8	0
Sum n-7	311	2.9	1.8–4.4		306	3.3	2.0–4.4	
18:1n-9	311	8.8	6.8–11.2	0	306	11.5	7.9–16.4	0
20:1n-9	311	0.36	0.06–3.9	0	306	0.49	0.13–9.4	0
20:3n-9	311	0.33	0.02–2.6	0	306	2.6	0.46–5.8	0
22:1n-9	311	0.04	0.0–0.20	0	306	0.07	0.0–0.20	0
24:1n-9	311	3.7	2.4–5.7	0	306	3.9	2.3–5.6	0
Sum n-9	311	13.5	9.7–20.2		306	18.8	11.1–26.0	
TT18:2n-6	311	0.07	0.0–0.25	0	306	0.09	0.0–0.30	0.3
TC18:2n-6	311	0.01	0.0–0.17	13.8	306	0.02	0.0–0.20	13.7
CT18:2n-6	311	0.02	0.0–1.1	0.3	306	0.04	0.0–2.1	0.3
PT16:1n-7	311	0.50	0.11–1.2	0	306	0.42	0.06–1.1	0
PT18:1n-9/7	311	0.06	0.01–0.29	0	306	0.05	0.0–0.25	0
Sum trans	311	0.70	0.27–1.9		306	0.67	0.35–2.8	
C18 trans	311	0.17	0.04–1.4		306	0.22	0.04–2.3	
MUFAs	311	16.8	12.5–21.3		306	19.9	13.7–26.0	
LCPUFAs	311	32.2	20.2–36.5		306	28.8	20.7–34.1	

Values are percentage by weight. SAFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.

TABLE 5. Correlations between the fatty acid composition (% by weight) of the umbilical vein and the Neurological Optimality Score (NOS) at 18 months

	Neurological Optimality Score			
	Univariate analysis		Multivariate analysis	
Vein	Spearman's rho	P-value	Standardized coefficient	P-value
Saturated	-	N.S.	-	NS
Monounsaturated	-	N.S.	-	NS
Omega-6				
20:4n-6	0.13	0.03	-	NS
Omega-3				
22:6n-3	-	N.S.	-	NS
Total *trans* fatty acids	-0.23	<0.0005	-0.52	0.03
C18-*trans* fatty acids	-0.19	0.0001	-0.16	0.02
Trans-18:1n-9 or 7	-0.12	0.04	-	NS
Trans,cis-18:2n-6	-0.21	<0.005	-0.20	0.001
Trans-16:1n-7	-0.12	0.048	-0.14	0.02

Factors which played an additional significant role in at least one of the multivariate models were education of the father, Home Observation for Measurement of the Environment score, weight of gravida before pregnancy, uncertain or unreliable date of last menstrual period, breastfeeding for more than 6 weeks, and age of assessment at 18 months. Allocation into control formula or LCPUFA-supplemented formula group and primi- versus multiparity did not affect NOS significantly. NS, not significant.

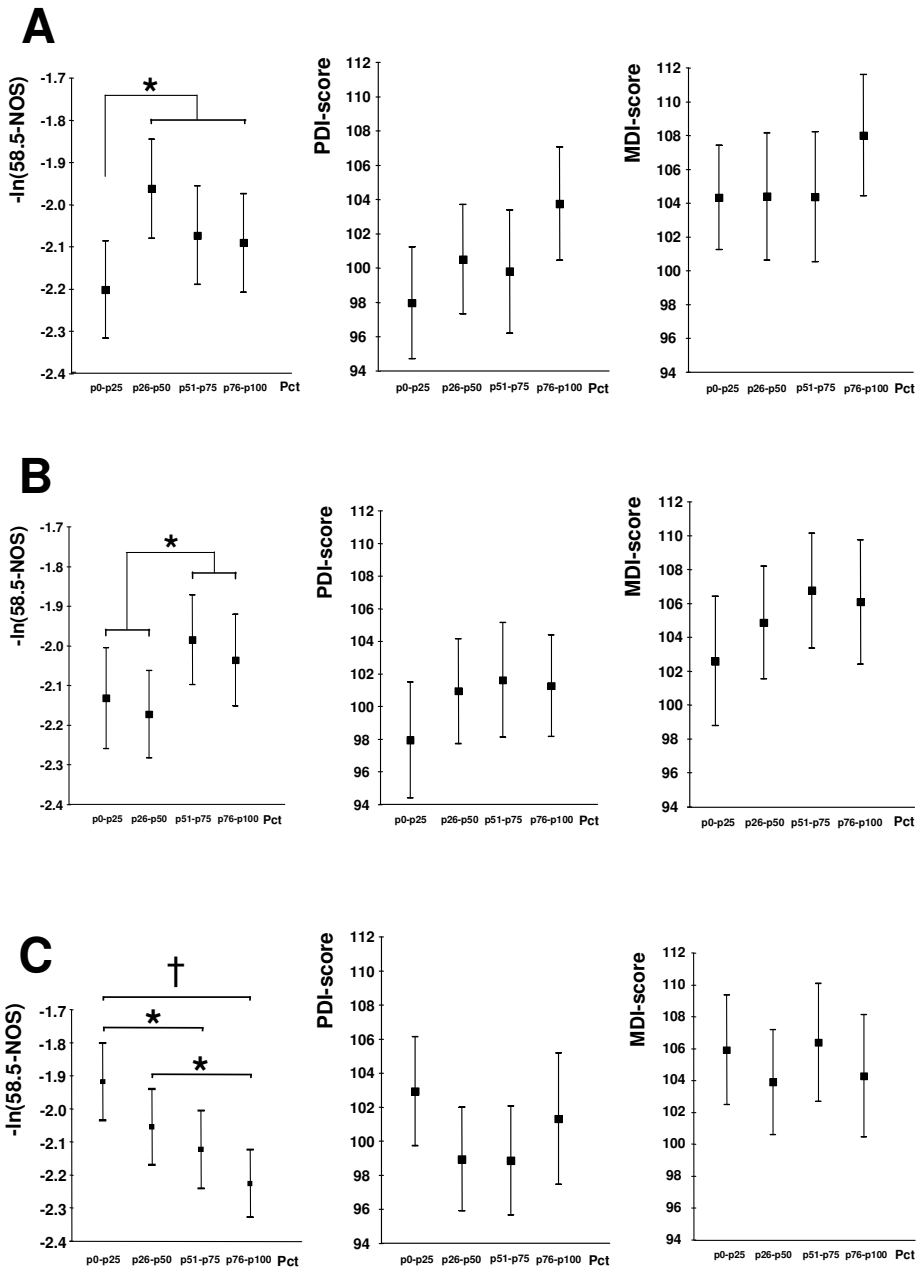

FIGURE 2. Relationships between umbilical vein content of DHA (A), AA (B) and the sum of *trans*-fatty acids (C) expressed in quartiles and NOS and PDI and MDI scores. The NOS is expressed in the transformed NOS, which was used to achieve Gaussian distribution. Note that a higher –ln(58.5 – NOS) value means a better NOS. The *squares* denote mean values, the *vertical bars* the 95% confidence interval of the mean. *Asterisks* denote statistically significant differences between groups in the univariate analyses (Mann-Whitney U test): *$p < 0.05$, † $p < 0.001$.

Discussion

The present study indicated that, in healthy term infants, a DHA content in the umbilical vein within the lowest quartile and a higher content of venous *trans*-fatty acids were associated with a less optimal neurologic condition at 18 mo. This effect was found with the Hempel technique but could not be demonstrated with psychomotor or mental development indices of the Bayley scales. This underscores the notion that the Hempel technique is a more sensitive instrument to assess possibly subtle effects of early nutritional condition on brain function than the Bayley scales, which are designed to detect major developmental delays (23). Our study indicates that low prenatal venous umbilical DHA content is associated with a less optimal neurologic condition at 18 mo. In the same study cohort as in the present study, we previously found that DHA and AA content in the umbilical vein was associated with a more optimal neurologic condition on postnatal d 10–14 (8). In addition, we found that neonatal AA content in the umbilical artery was related to a better neurologic condition at 3 mo, whereas DHA affected neurologic condition at 3 mo to a marginal extent (9). At 18 mo of age, we found the opposite: neonatal DHA content of the umbilical cord showed a significant correlation with neurologic optimality, whereas neonatal umbilical AA content only had a marginal effect on neurologic outcome. Taken together these findings suggest that both neonatal AA and DHA status might affect neurologic development to a limited extent. This could explain why previous studies produced conflicting results. Two studies that evaluated cognitive function in similarly large populations of full-term infants could not establish a relationship between neonatal LCPUFA status and cognitive function at 4 and 7 y (10,11). On the other hand, two supplementation studies with DHA during pregnancy found that a higher neonatal DHA status was positively associated with more mature electroencephalographic scores on the second day of life and latencies to visual evoked potentials recorded to pattern-reversal stimuli at 10 wk and 6 mo (29,30). Previous studies indicated that postnatal supplementation with DHA affects neurologic condition during early infancy, but not neurodevelopmental outcome at 1–3 y (24,31,32). In the present population, we also did not find an effect of breast-feeding or LCPUFA-enriched formula on neurologic condition at 18 mo (24). The present study demonstrated that the association of prenatal DHA with neurodevelopmental outcome was not restricted to early infancy, but was still demonstrable at the age of 18 mo (8,30,33). Although the association was relatively small, it remained present when we took into account the effect of postnatal LCPUFA supplementation. This suggests that in terms of neurologic health prenatal DHA status is more important than postnatal DHA status. This underscores the notion that we should pay attention to the essential fatty acid status of pregnant women, in particular of multiparous women (34,35). The association between venous umbilical DHA content and NOS was not a linear one, but showed a threshold effect. Animal studies suggest that low DHA status results in deviant

behavior. n-3 LCPUFA–deficient rhesus monkeys showed more stereotyped motor behavior than control animals fed a matched control diet abundant in n-3 fatty acids (36). In rats, low brain content of DHA induces altered dopaminergic function and behavior (37). Surprisingly, we found that the venous umbilical *trans*-fatty acid content was more prominently associated with neurodevelopmental outcome at 18 mo than LCPUFA content, an association that was statistically independent of DHA and AA content of the umbilical vein. This negative association remained statistically significant when we excluded the breastfed infants from the analyses to evaluate whether the association still persists without potential postnatal *trans*-fatty supply *via* breast-feeding. Before addressing the putative biologic mechanisms underlying this finding, we emphasize that we cannot exclude that a certain but small amount of all *trans*-fatty acids present were not analyzed, *e.g.* the longer chain *trans*-fatty acids. Thus, we found a negative correlation between the lion's share of *trans*-fatty acids and neurologic condition at 18 mo. Because *trans*-fatty acids cannot be synthesized *de novo*, the content of *trans*-fatty acids in the umbilical wall reflects maternal dietary intake of *trans*-fatty acids. *Trans*-fatty acid can potentially alter numerous cell membrane properties when incorporated in membranes (38); however, no significant *in vivo* incorporation of *trans*-fatty acids in the neuronal membranes takes place (16). Despite the minor effects of *trans*-fatty acids on the fatty acid composition of neuronal membranes, high *trans*-fatty diets do induce dopaminergic alterations in the brains of pigs and rats (39,40). The biochemical mechanisms are not yet clear, but it seems unlikely that the biochemical effects of *trans*-fatty acids are mediated by neuronal membrane function. It has been shown that fatty acids can alter gene transcription that impacts lipid, carbohydrate, and protein metabolism as well as cell growth and differentiation *via* several transcription factors in the cytoplasm and cell nucleus (4). It could be that some specific *trans*-fatty acids such as conjugated *trans*-fatty acids by means of interaction with peroxisome proliferator–activated receptors (PPARs) might induce unfavorable neuronal function. To our knowledge, no data are available on the effects of *trans*-fatty acids on neurodevelopmental outcome (14,16). Such knowledge is urgently needed as it is possible that humans are more vulnerable to early *trans*-fatty acid exposure than animals. This is suggested by the finding that *trans*-fatty acids do not affect birth weight, growth, and longevity in animals (14,41), whereas in human preterm infants, *trans*-fatty acids are negatively correlated to birth weight (12). Besides the direct potential harmful effects of *trans*-fatty acids on neurodevelopment, we originally assumed that *trans*-fatty acids could also be indicators of the presence of less favorable dietary constituents in general, which in turn could be associated with a lower socioeconomic status. However, our data indicated that higher *trans*-fatty content to a limited extent was even related to a higher social class as indicated by the father's profession (Table 2). A recent report of the Dutch Ministry of Health, Welfare, and Sports indicated that the intake of *trans*-fatty acids among Dutch young adults was not related to socioeconomic characteristics (17). These findings do not exclude the possibility that a higher *trans*-fatty acid intake may be associated with an unhealthy diet or

lifestyle. To illustrate the point, the consumption of a diet rich in *trans*-fatty acid content has been associated with bakery products, confectionery, and snacks (42). In conclusion neonatal DHA status, used as a proxy of maternal supply during pregnancy, has a small but statistically significant beneficial effect on neurologic condition at 18 mo. In addition, neonatal *trans*-fatty acid status has a substantial negative effect on neurologic condition at 18 mo. The latter finding might support a plea for the removal of the industrially produced *trans*-fatty acids from our diet.

Acknowledgments.
We gratefully acknowledge the statistical advice of Dr. M. R. Fokkema.

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General Discussion

4

4 Discussion

4.1 The influences of postnatal feeding on neurodevelopment

The central question of the first part of this thesis is whether formula feeding supplemented with long chain polyunsaturated fatty acids (LCPUFAs) has a positive effect on neurodevelopmental outcome of healthy term infants. Our double blind randomized trial indicated that there is a beneficial effect of LCPUFA supplementation on the quality of general movements at 3 months but not on neurodevelopmental outcome at 18 months of age. The importance of LCPUFAs in the functioning of the nervous system is widely acknowledged[1,2]. This does not necessarily imply that formula feeding should be supplemented with LCPUFAs. Double blind randomized trials can give the highest possible evidence of the effectiveness of LCPUFA-supplementation on neurodevelopment. We have used three different neurodevelopmental assessments to detect functional and qualitative differences in neurodevelopmental outcomes at 3 and 18 months.

4.1.1 Differences between formula groups

The quality of general movements was assessed at 3 months. Test results are classified into definitely abnormal, mildly abnormal, normal suboptimal and normal optimal general movements[3]. Definitely abnormal general movements are associated with a high risk for cerebral palsy and complex minor neurological dysfunction. The latter can be considered as borderline cerebral palsy[3]. None of the infants described in this thesis had definitely abnormal general movements, which confirms that we investigated a healthy term population of infants. However, twenty-five percent of all infants showed mildly abnormal general movements, which can be considered as a risk factor for the development of minor neurological dysfunctions, attention problems and behaviour problems at school age, as has been demonstrated in a mixed population of infants with a low and a high risk for developmental problems[3,4]. Unfortunately, until now no data is available regarding the significance of mildly abnormal general movements in a population with a low risk for developing neurodevelopmental disorders. Therefore, follow-up of the current cohort of infants is needed to confirm possible long-term consequences of mildly abnormal general movements on later development.

The Bayley Scales at 18 months were used to detect delays in development. The Bayley scales have been designed to detect clinical developmental delays but are not sensitive enough to detect subtle differences in neurodevelopment. Furthermore, some large studies have demonstrated that the concurrent validity and the predictive validity of the Bayley Scales are limited[5,6]. This can partly be explained by the large variance in the measurements caused by inherent biological variability of expression of neurodevelopmental milestones. At the age of 18 months, we also used the sensitive neurological examination according to Hempel to assess developmental milestones but apart from that also the quality of motor performance[7,8]. Although developmental milestones can be assessed more objectively and quickly than the assessment of the quality of motor behaviour, the examination according to Hempel has proven its usefulness in the detection of small differences in studies investigating the effects of polychlorinated biphenyls (PCBs)[9,10]. At present, there is no data available about the (predictive) validity of the examination according to Hempel.

The findings of our randomized trial are in line with similar trials done by others investigating the effects of LCPUFA supplementation. The review of similar LCPUFA-

trials described in the introduction of this thesis (chapter 1 §1.3.3) showed that the effects of LCPUFA supplementation tended to be subtle and transient[11]. Several other studies have also found a beneficial effect of LCPUFA supplementation on neurological function around the age of 3 months. One study showed a beneficial effect on the Brunet-Lezine Developmental Quotient at 4 months and several other studies found a favourable effect on visual function at 3 to 4 months of age[12-15]. Randomized controlled trials that used developmental outcomes beyond the age of 4 months indicated little or no beneficial effect of LCPUFA supplementation[11,16]. It must be stressed that it is inherently difficult to detect differences in neurological condition because of large physiological variations of motor behaviour[17]. Especially at the age of 6-24 months a 'latency' of expression of minor neurological dysfunction takes place which makes the detection of minor neurological signs complicated, because most minor neurological dysfunction manifest itself only when more established complex neural functions develop at school age[11,18]. At present, no LCPUFA supplementation studies have been published using long-term follow up assessments beyond the age of 24 months. The possibility that beneficial effects of LCPUFA supplementation are expressed at later ages can not be excluded, because many 'latent' minor neurological signs are being expressed at school age, i.e. at an age at which complex neuronal circuitries become functionally active[18]. In summary, postnatal supplementation of LCPUFAs for two months induces subtle beneficial effects on the neurological condition at 3 months but not at 18 months.

4.1.2 Differences between formula fed and breastfed infants

When breastfed infants are compared with infants receiving formula feeding with or without LCPUFAs, we found that breastfed infants have less mildly abnormal and more optimal general movements at 3 months. A subgroup analysis of the breastfed group revealed that breastfeeding for more than 6 weeks was associated with more optimal and less mildly abnormal general movements. These findings were adjusted for obstetrical, social-economical status and other environmental circumstances by means of the Home Observation for Measurement of the Environment score (HOME)[19] to find that they remained statistically significant. Another alternative explanation for the observed beneficial effect of breastfeeding could be that infants who have a more favourable neurological condition are more likely to be breastfed for longer durations. Although the latter two explanations were not supported by a study of Lucas and Morley who found that the IQ of children who had been fed human milk by nasogastric tube was 8 points higher at 8 year compared to children who received formula by nasogastric tube[20]. It is of importance to note that the children in this study were not randomized with respect to breast or formula grouping. To prove that breastfeeding induces beneficial effects, double blind randomized trials must be performed. However, randomized controlled trials on breastfeeding are not ethically justified. Recently, it became clear that maternal IQ explains a great deal of the observed beneficial effects of breastfeeding on cognitive development compared with formula fed infants[21]. Some studies indicate that breastfeeding is associated not only with a better cognitive development but also with a somewhat better neurodevelopmental outcome[10,22,23]. For instance, Lanting et al. found that infants who received $\geq$ 6 weeks breastfeeding had more fluent movements at the age of 42 months[10]. At the age of 18 months we could no longer find a beneficial effect of breastfeeding which could be explained by the 'latency' of expression of minor neurological signs at this age as has been described earlier in this section[17]. Our negative study results at 18 months are in line with a similar large randomized trial of Auestad et al. 2001 who also showed no effect of

breastfeeding on neurodevelopmental outcome at 18 months[24]. To summarize, breastfeeding for more than 6 weeks was associated with a better neurological condition at 3 months but not at 18 months.

4.2 Prenatal fatty acid status and neurodevelopment

The second part of this thesis deals with the question whether prenatal LCPUFA status affects neurodevelopmental outcome. Prenatal fatty acid status was associated with neurodevelopmental outcome at birth and at the ages 3 and 18 months after term, an effect that was independent of type of postnatal feeding. To assess the relationship between intrauterine fatty acid status and neurodevelopmental outcome we used the measurement of the fatty acid composition of the walls of the umbilical vessels as a proxy for prenatal fatty acid status of infants[25]. Note that information is not yet available about the relationship between the fatty acid composition of the umbilical vessels and the brain. However, Markrides et al.[26] provided evidence that erythrocyte DHA status of infants aged less than 48 weeks correlated with the DHA composition of the brain. Because the umbilical vein LCPUFA status is likely to be related to the concurrent prenatal erythrocyte LCPUFA status, it is plausible that umbilical LCPUFA status is also a valid marker of the LCPUFA content of the brain[25]. Indeed, animal studies have indicated that dietary alterations in LCPUFAs during the growth spurt induce differences in both peripheral tissue and cerebral cortex LCPUFA composition that in turn induces changes in neurodevelopmental outcome[1,27]. The relationships between the peripheral LCPUFA status and the LCPUFA contents of the brain seem to vanish beyond the age of 2 years at which age the growth spurt ends[1]. A study of Carver et al.[28] indicated that the fatty acid composition of erythrocytes measured at autopsy is not a reliable predictor of the fatty acid composition of the cerebral cortex of children aged 2 to 18 years.

Dijck et al[29]. demonstrated a positive relationship between prenatal LCPUFA status and the neurological condition immediately after birth in the groups studied in the present thesis. At 3 months, we found that infants showing mildly abnormal general movements had a somewhat less favourable prenatal LCPUFA profile compared to the infants with normal quality of general movements. The observed differences in LCPUFA status between normal and mildly abnormal general movements were small but statistically significant.

At 18 months we found that prenatal DHA and AA status showed a positive relationship with neurological condition as has been measured with the neurological optimality score (NOS) of the Hempel assessment. However, the positive relationship between AA and neurological condition did not retain its significance after correction for confounders. Of importance is to note that there was no linear relationship between the DHA status in the umbilical vein and the NOS. Infants who had DHA status within the lowest quartile, showed a less favourable NOS than infants with a higher DHA status. Therefore, it seems that a minimal threshold amount of DHA supply from the mother is necessary for optimal neurological condition at 18 months.

Remarkably, supplementation of LCPUFAs or breastfeeding did not modify the observed associations between prenatal fatty acid status and neurodevelopment at birth and at 3 and 18 months in the multivariate analyses. This supports the notion that prenatal LCPUFA supply is more important than postnatal feeding. There were no significant interaction effects of prenatal LCPUFA status and type of postnatal feeding on neurological condition at 3 and 18 months. Therefore, apart from the evaluation of the effects of postnatal feeding

on neurodevelopment, also the relationships between the prenatal LCPUFA status and neurological outcome can be studied in this thesis.
We found that the relationship between prenatal LCPUFA status and the quality of general movements at 3 months only was related with the LCPUFA status in the umbilical arteries, carrying the LCPUFAs away from the foetus to the placenta. The umbilical artery fatty acid composition is positively affected by placental supply and fetal synthesis and negatively influenced by fetal extraction of fatty acids. We did not find any differences in umbilical vein fatty acid composition between infants with normal GMs and mildly abnormal GMs. This might imply that an inadequate maternal fatty acid supply was not a determinant of the quality of GMs in this study population. Instead, our data suggest that the development of mildly abnormal GMs might be associated particularly with the impaired extraction or increased dilution of AA and EFA driven by fetal metabolism and not by maternal supply. It could be that dilution of EFA and LCPUFAs in the umbilical artery is the result of an increased maternal supply of glucose to the placenta that induces extra *de novo* fatty acid synthesis from glucose, especially saturated fatty acids and MUFAs in the fetus.
A more detailed inspection of the relationships between prenatal DHA and AA status and neurodevelopment revealed that a higher AA status was more related with the early neurological condition (at birth and at 3 months) than with the neurological condition at 18 months. It should be kept in mind that only preliminary conclusions can be drawn from these aforementioned results regarding the relationship between prenatal LCPUFA status and neurological condition. More measurements in time are needed to trace the maternal LCPUFA status during gestation to draw definite conclusions regarding the relationship between the prenatal LCPUFA status and neurodevelopmental outcome of the foetus or infant. Double blind randomized trials in which maternal supplementation of LCPUFAs during pregnancy improve the LCPUFA status of the foetus can give also valuable insights about the effects of improved LCPUFA status on neurological development[30].
A secondary aim of the analysis was to study the potential negative effects of *trans* fatty acids on neurodevelopment. Decsi and other members of the LCP-project had already found a negative relationship between the *trans* fatty acid content and LCPUFA status in the umbilical vein in the population studied in this thesis[31]. Indeed, *trans* fatty acids were negatively related with the neurological optimality score at 18 months. The relationship between *trans* fatty acids and neurodevelopmental outcome was even stronger than that between the LCPUFA status and outcome. Multivariate regression analyses confirmed the association which also revealed that the association was independent of LCPUFA status. This indicates that the presumably negative effects of *trans* fatty acids on neurodevelopment at 18 months can not only be explained by the decrease of the LCPUFA contents in the umbilical vessel lipids. Overall, the results are to be interpreted with caution because we report associations which are not cause-effect relationships between prenatal fatty acid status and neurodevelopment. Therefore these findings should be confirmed by other future studies (e.g. by randomized controlled trials). To illustrate this point, *trans* fatty acids may be a marker for an unhealthy lifestyle. However, in our study low socioeconomic status was not related with *trans* fatty acid intake in our study which is also in line with a recent report of the Dutch Ministry of Health, Welfare, and Sports[32]. Since endogenous *trans* fatty acids synthesis is not possible, the prenatal *trans* fatty acid content in the umbilical wall is a representation of the dietary intake of *trans* fatty acids by the mother. These findings suggest that avoidance of the intake of *trans* fatty acids could prevent a less favourable neurological condition at 18 months. High quality studies are

urgently needed to confirm the relationship between maternal *trans* fatty acid intake and less favourable neurological condition.

4.3 Perspectives on future research

The effects of LCPUFA supplementation on neurodevelopmental outcome may be detected when large randomized trials are being performed with relatively high concentrations of LCPUFAs, in particular DHA (≥ 0.30%) and with little attrition at follow up. Potentially, subtle effects are more likely to be found in qualitative differences in motor development than in motor milestone achievements. Long-term follow-up of our study cohort is currently performed at school age at which age we are able to detect potential beneficial effects of LCPUFAs on complex motor and cognitive tasks.

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Summary

5

5 Summary

5.1 Introduction

Long-chain metabolites of the parent essential fatty acids called long-chain polyunsaturated fatty acids (LCPUFAs) are major membrane components in the central nervous system. Dietary intake partly determines the availability of LCPUFAs as building blocks for neuronal structures. Fifty till sixty percent of the dry brain weight consists of fatty acids of which about twenty percent are LCPUFAs. In particular two LCPUFAs, docosahexaenoic acid (DHA) and arachidonic acid (AA) are being incorporated into neuronal tissue. Conversion of the essential fatty acid alpha-linolenic acid into DHA is inefficient in humans, so DHA status is relatively dependent on dietary intake of DHA, which is currently low in western societies. Many studies have provided evidence that DHA and AA play a role in adequate functioning of the central nervous system. During the so-called brain growth spurt, which takes place shortly before and after birth until about the age of 2 years, large amounts of fatty acids are being incorporated into the nervous system. It is therefore remarkable that conventional formula feeding does not contain LCPUFAs, whereas breastfeeding does contain LCPUFAs. LCPUFAs could partially explain the observed positive effect of breastfeeding on neurodevelopment.

To study the potential beneficial effects of LCPUFAs and the type of early postnatal feeding on neurodevelopmental outcome we investigated 472 healthy term infants in a double blind randomized controlled trial with a breastfed reference group. Follow-up after two months of LCPUFA supplementation was conducted at the ages of 3 and 18 months to evaluate neurodevelopmental outcome. In addition, we evaluated the relationships between prenatal LCPUFA status and the neurodevelopmental outcome at 3 and 18 months in the same study cohort. For this we collected samples of the umbilical vein and artery for the determination of the LCPUFA content. The LCPUFA content was used as a marker of prenatal LCPUFA status (See also Chapter 1, figure 5).

5.2 Is postnatal LCPUFA supplementation beneficial for neurodevelopment?

The addition of LCPUFAs to standard formula for the duration of two months did improve the quality of general movements (GMs) at the age of 3 months (Chapter 2 §2.1). The evaluation of the GMs has proven to be a sensitive technique for evaluating the quality of brain function in young infants. The quality of GMs was classified into normal optimal, normal suboptimal, mildly abnormal and definitely abnormal. No definitely abnormal GMs were observed, which is in agreement with data from a healthy term study population. A significant reduction in the presence of mildly abnormal general movements was observed in the LCPUFA supplemented formula group compared with the control formula group. When expressed as ‘numbers needed to treat’, eight infants have to supplemented with LCPUFA to prevent the occurrence of one infant with mildly abnormal GMs. Breastfed infants showed more optimal GMs compared with the formula-fed groups after correction for confounders. To investigate the minimal duration of exclusive breastfeeding for optimal neurological condition at 3 months, a subgroup analysis in the breastfeeding group was performed. The median duration of breastfeeding was 9 weeks. Inspection of the association between the duration of exclusive breastfeeding and the quality of GMs revealed that there was a positive association between the duration of exclusive

breastfeeding and the quality of GMs. Close inspection of the raw data revealed that a saturation effect occured at the age of ≈6 weeks. Infants who had received breastfeeding for more than 6 weeks had more optimal GMs and less mildly abnormal GMs at 3 months (Chapter 2 §2.2). Our study could not answer the question whether breastfeeding for longer periods would be even better for the neurological condition, as only few mothers breastfed their infant for more than 12 weeks and because we assessed outcome as early as 3 months.
The beneficial effect of LCPUFA supplementation at the age of 3 months was no longer observed at the age of 18 months as measured by the Bayley Scales and the neurological examination according to Hempel. Breastfeeding also did not enhance neurodevelopmental outcome at 18 months.
To summarize, it seems that the beneficial effects of LCPUFA supplementation are present at 3 months and disappear prior to the age of 18 months at which age it is notoriously difficult to find differences in motor and cognitive performance. The absence of difference between the feeding groups however does not preclude the finding of differences at later ages when complex neurological circuitries have become functionally active. Therefore, a follow up at school age is currently performed to evaluate the long-term effects of LCPUFA supplementation in the same study population.

5.3 Prenatal fatty acid status and neurodevelopmental outcome

In addition to the evaluation of the influences of postnatal feeding on neurodevelopmental outcome, we assessed the influences of prenatal nutrition by measuring the fatty acid composition of the umbilical vessels shortly after birth. Chapter 3 describes the associations between the fatty acid composition of the umbilical vessels, a proxy of prenatal fatty acid status, and the neurodevelopmental outcomes at 3 and 18 months. Infants showing mildly abnormal GMs had a lower AA, DHA and essential fatty acid status in the umbilical artery (Chapter 3 §3.1). Therefore, infants with mildly abnormal GMs could have a less favourable LCPUFA profile at birth than infants with normal GMs.
No correlations were found between the prenatal fatty acid status and the Bayley score at 18 months. However, the more sensitive neurological examination according to Hempel revealed that the fatty acid composition of the umbilical vein correlated with the neurological optimality score at 18 months. Infants with an umbilical vein DHA status within the lowest quartile had a lower neurological optimality score compared to infants with a higher umbilical DHA status. The mean effect size was about 1-2 points on a scale with a maximum of 57 points. The significant association between arachidonic acid status and the neurological optimality score disappeared when correcting for confounding factors. The latter finding confirms that the observed relationships between prenatal LCPUFA supply and neurodevelopmental outcome are subtle at best.
Apart from the relationship between prenatal LCPUFA status and neurodevelopment, we were also interested in the potential harmful effects of *trans* fatty acids on neurological development because of the reported inhibitory effect of *trans* fatty acids on LCPUFA synthesis in the literature. Surprisingly, we found that prenatal *trans* fatty acids exhibited an even stronger negative relationship with the neurological condition at 18 months than prenatal LCPUFA status. The mean difference in the neurological optimality score between the lowest and the highest quartile of *trans* fatty acid content was about 3 points. This means that infants who are exposed to relatively high levels of *trans* fatty acids *in utero* was associated with a somewhat less quality of motor behaviour which for instance can

become manifested by a lesser ability to avoid objects, less fluent movements and less variations in motor behaviour.
Remarkably, our results indicated that prenatal nutritional influences on neurodevelopment were stronger than influences of type of postnatal feeding, i.e. either breastfeeding or infant formula feeding.

5.4 Conclusions

The development of the infant is a complex interplay between genetics and environment in which LCPUFA status plays a role. The following conclusions can be drawn from the studies described in this thesis:

- LCPUFA supplementation (0.30% DHA and 0.45% AA) in formula feeding for two months significantly reduces the occurrence of mildly abnormal general movements at the age of 3 months in a population of healthy term infants;
- Infants who received breastfeeding for more than 6 weeks had less mildly abnormal general movements at 3 months compared with infants who received breastfeeding for 6 weeks with or without correcting for confounding factors;
- Infants who received breastfeeding or LCPUFA supplemented formula feeding had a similar neurodevelopmental outcome at 18 months as measured with the Hempel assessment and the Bayley Scales compared with the standard formula fed infants;
- Prenatal LCPUFA status and *trans* fatty acid content was related with neurodevelopmental outcome at 3 and 18 months even when type of postnatal feeding was taken into account;
- Infants who had mildly abnormal general movements at 3 months had a less favourable prenatal LCPUFA status;
- Prenatal DHA status within the lowest quartile was associated with a less favourable quality of motor behaviour at 18 months;
- Prenatal *trans* fatty acid content was negatively associated with the quality of motor behaviour at 18 months.
- Prenatal fatty acid status might be more important as a determinant for neurological condition than type of postnatal feeding (breastfeeding, LCPUFA or standard formula feeding).

Dankwoord

6

6 Dankwoord

Alvorens het dankwoord op te schrijven begin ik eerst met een korte inleiding over hoe ik in het zogenaamde MD/PhD promotietraject terecht ben gekomen. De keuze voor de studie geneeskunde was vooral gebaseerd op mijn belangstelling in de combinatie tussen wetenschap en het helpen van mensen. Gaandeweg de studie viel mij op dat de wetenschappelijke facetten van het boeiende beroep van medicus minder aan bod kwamen. Tijdens het neurologie-onderwijs in het tweede studiejaar kreeg ik een aantrekkelijk aanbod van prof. Mijna Hadders-Algra om naast de colleges zelfstandig onderzoek te doen in het kader van het LCP-project. Graag zou ik daarom allereerst Mijna Hadders-Algra hartelijk willen bedanken voor de grote steun en praktische begeleiding vanaf het begin tot het einde van mijn promotie. Mijna haar grote betrokkenheid en altijd aanwezige bereidheid om te onderwijzen, discussiëren en te adviseren waardeer ik zeer. Zelfs s'avonds en in het weekend kreeg ik antwoord op mijn emails! Mijn belangstelling voor het doen van wetenschappelijk onderzoek is daardoor sterker geworden en heeft zelfs geresulteerd in de voortzetting van onderzoek buiten het proefschrift om naar de implementatie en de voorspellende waarde van de beoordeling van de spontane motoriek van pasgeborenen op het consultatiebureau.
Prof. Frits Muskiet van de afdeling Laboratoriumgeneeskunde & Pathologie en prof. E.R. Boersma (Kindergeneeskunde) zijn van begin af aan als promotoren sterk betrokken geweest bij het tot stand komen van het promotieonderzoek en hebben gezorgd dat het promotieonderzoek met recht multidisciplinair genoemd mag worden. De internationale samenwerking met prof. Tamás Decsi en prof. Günther Boehm is heel goed uitgepakt. De analyse van de vetzuren in de navelstreng werd door Tamás Decsi uitgevoerd in het laboratorium van het ziekenhuis van Pecs, Hongarije. Dankzij de expertise van Tamás Decsi hebben wij de transvetzuren kunnen meten in de navelstrengen en de negatieve effecten daarvan op het kind gevonden welke met de standaard analyse methodes in Nederland anders niet waren ontdekt. Günther Boehm heeft er o.a. mede voor gezorgd dat we Europese subsidie konden verkrijgen voor de follow-up van de kinderen op 9-jarige leeftijd uitgevoerd door Corina de Jong.
De regelmatige bijeenkomsten van de LCP-werkgroep die bestond uit E.R. Boersma, Janneke Dijck, Mijna Hadders-Algra, Frits Muskiet, en mijzelf zijn bijzonder vruchtbaar geweest. De wetenschappelijke discussies n.a.v. de resultaten waren niet alleen interessant maar ook amusant. Het plezier in het uitvoeren van onderzoek is hierdoor gestimuleerd. Hartelijk dank hiervoor. Ik kan iedere toekomstige promovendus trouwens aanraden om regelmatig met alle promotoren te discussiëren over de voortgang en uitkomsten van het promotieonderzoek!
Hierbij wil ik de leescommissie bedanken voor de moeite om het manuscript goed door te lezen. De leescommissie bestond uit prof.dr A.F. Bos, prof.dr B. Koletzko en prof.dr H.N. Lafeber.
De voorzitter van de 'Junior Scientific Masterclass' (JSM), prof. T.H. The wil ik bedanken voor het aanbieden van de mogelijkheid om mijn promotieonderzoek en de studie geneeskunde in acht jaar te voltooien.
Alle collega's van de afdeling Ontwikkelingsneurologie functioneren als één team waarbij er veel voor elkaar gedaan wordt. Daarom wil ik de volgende collega-aio's bedanken: Cornill Blauw-Hospers, Tineke Dirks, Saskia van Goor, Kirsten Heineman,

Corina de Jong, Ramses Kemperman, Karin Middelburg en Lieke Peters. Veel succus met het afronden van jullie proefschrift!

Graag wil ik Annemarie Borsboom en Loes de Weerd bedanken voor de secretariële ondersteuning.

Om de gegevens goed te interpreteren waren soms lastige statistische methodes en modellen nodig. Graag zou ik daarom dr. V. Fidler willen bedanken voor de belangeloze waardevolle statistische adviezen. Het maken van de regressiemodellen heb ik geleerd van Rebecca Fokkema waar ik haar zeer dankbaar voor ben. Ook wil ik Leo van Eykern bedanken voor de stimulerende en adviserende rol bij de interpretatie van de gegevens en voor de goede (politieke) discussies tijdens de koffiepauzes. Graag zou ik tevens prof. Ab Gramsbergen en al diegenen die altijd tijdens de Ontwikkelingsneurologie toogdag geïnteresseerd waren in de vorderingen van mijn promotieonderzoek bedanken voor hun interesse en feedback.

Last but not least wil ik alle deelnemers aan het onderzoek bedanken voor hun belangeloze deelname aan het onderzoek!

Samenvatting

7

7 Samenvatting

Inleiding

Het onderzoek is gestart met als verwachting dat de ontwikkeling van kinderen beter wordt wanneer er zogenaamde lange keten meervoudig onverzadigde vetzuren (LCPUFAs) aan de standaard flesvoeding toegevoegd worden. Het is namelijk al geruime tijd bekend dat vetzuren belangrijke voedingsstoffen zijn voor het zich ontwikkelende kind. Deze vetzuren worden als bouwstenen gebruikt in de hersenen. Vooral de vetzuren die afgeleid zijn van de essentiële vetzuren worden in hoge hoeveelheden in de hersenen ingebouwd. Deze afgeleide vetzuren worden lange keten meervoudig onverzadigde vetzuren genoemd. De belangrijkste lange keten meervoudig onverzadigde vetzuren zijn arachidonzuur en docosahexaeenzuur (DHA). Arachidonzuur zit vooral in vlees en DHA zit vooral in vette vis, zoals makreel. Het kind krijgt voor de geboorte deze vetzuren via de moederkoek binnen en na de geboorte via de borstvoeding. Omdat er geen lange keten meervoudig onverzadigde vetzuren in standaard flesvoeding zitten maar wel in borstvoeding, rees de vraag of dit niet toegevoegd moet worden aan de flesvoeding. In dit proefschrift zal deze vraag centraal staan. Het tweede deel van het proefschrift gaat er over of er ook aanwijzingen zijn te vinden of de LCPUFA status, zoals gemeten in de navelstreng ten tijde van de zwangerschap belangrijk is voor de ontwikkeling van het kind.

Naast onderzoeken beschreven in dit proefschrift, zijn er vele andere onderzoeken gepubliceerd over het effect van lange keten meervoudig onverzadigde vetzuren in flesvoeding op de ontwikkeling van het kind. Bijna alle uitkomsten zitten op één lijn. Er is een tijdelijk positief effect op de motoriek en de gezichtscherpte van baby's aangetoond. Er zijn geen effecten van lange keten meervoudig onverzadigde vetzuren gevonden op de peuterleeftijd en er zijn geen gegevens bekend over de effecten op de ontwikkeling na de peuterleeftijd. Het ontbreken van een effect op de peuterleeftijd hoeft niet uit te sluiten dat er op latere leeftijd wel een effect aan te tonen is, omdat sommige aspecten van de ontwikkeling pas op latere leeftijd tot volledige uiting komen. Dus als er in werkelijkheid al een gunstig effect is dan is het tijdelijk, subtiel, en moeilijk te meten. Bedacht moet worden dat ontwikkeling heel moeilijk precies te meten is omdat er veel variatie in ontwikkeling is. De invloed van voeding op de ontwikkeling is op voorhand moeilijk aan te tonen omdat voeding maar een van de vele factoren is die de ontwikkeling beïnvloeden. In het onderzoek uit dit proefschrift werd getracht de neurologische ontwikkeling te meten met behulp van gevoelige meetinstrumenten, zodat we ook eventuele subtiele effecten op het spoor zouden kunnen komen.

De drie hoofdvragen die beantwoord worden in dit proefschrift zijn:

1 Wordt de neurologische en motorische ontwikkeling van het kind gunstig beïnvloed door flesvoeding waaraan meervoudig onverzadigde vetzuren zijn toegevoegd?
2 Is borstvoeding nog steeds het beste voor de ontwikkeling van het kind?
3 Bestaat er een verband tussen de voedingstoestand van het kind *voor* de geboorte d.w.z. de vetzuursamenstelling van bloedvaten van de navelstreng voor de geboorte en de neurologische en motorische ontwikkeling van het kind?

Positief effect van borstvoeding en flesvoeding met lange keten meervoudig onverzadigde vetzuren op de spontane motoriek op de leeftijd van 3 maanden

De kwaliteit van de spontane motoriek op de leeftijd van 3 maanden is een gevoelige maat voor de conditie van de hersenen van het kind. Borstgevoede kinderen die meer dan 6 weken borstvoeding hadden gekregen hadden mooiere bewegingen en minder vaak een licht afwijkende kwaliteit van de spontane motoriek dan de kinderen die flesvoeding hadden gekregen. In vergelijking met de standaard flesgevoede kinderen hadden de kinderen die lange keten meervoudig onverzadigde vetzuren in de flesvoeding hadden gekregen duidelijk minder licht afwijkende spontane bewegingen. Andere onderzoeken hebben aangetoond dat licht afwijkende spontane bewegingen mogelijk een risicofactor zijn voor onhandigheid en gedragsproblemen op de schoolleeftijd. De afname van het voorkomen van licht afwijkende bewegingen zou dus consequenties kunnen hebben voor de ontwikkeling op schoolleeftijd. Op de leeftijd van 18 maanden konden we geen effect van postnatale voeding meer zien op de ontwikkeling en het gedrag van het kind. Dit wordt bevestigd door soortgelijke andere onderzoeken, waarbij aangetekend moet worden dat lichte neurologische stoornissen zich niet zo gemakkelijk op deze leeftijd laten ontdekken. Op de schoolleeftijd openbaren zich namelijk veel meer lichte neurologische disfuncties dan op de peuterleeftijd, daarom worden de kinderen uit de huidige onderzoeksgroepen op de leeftijd van 9 jaar opnieuw onderzocht.

Is borstvoeding nog steeds het beste voor de ontwikkeling van het kind?

Al vroeg in de vorige eeuw werd erkend dat borstvoeding geven voor het kind beter is voor de ontwikkeling en groei dan flesvoeding geven. De flesvoedingsindustrie heeft er dan ook altijd naar gestreefd om haar flesvoeding zo veel mogelijk op die van de samenstelling van borstvoeding te laten lijken. Tegenwoordig worden er in sommige soorten flesvoeding behalve essentiële vetzuren ook lange keten meervoudig onverzadigde vetzuren toegevoegd, omdat laatstgenoemde niet in flesvoeding (bestaande uit koemelk én plantaardige vetten) zitten maar wel in borstvoeding. Maar is het wel echt zo dat de verschillen in ontwikkeling tussen borstgevoede en flesgevoede kinderen alleen toe te schrijven zijn aan het verschil in samenstelling van de voeding? Uit ons onderzoek bleek bijvoorbeeld dat de moeders die borstvoeding geven iets ouder zijn, minder roken, een hogere opleiding hebben en meer alcohol drinken tijdens de zwangerschap dan moeders die ervoor kiezen om flesvoeding te geven aan hun kind. Mogelijk heeft borstvoeding geven ook een gunstig effect, omdat de moeder-kind binding op die manier wordt vergroot. In ieder geval is het zo dat kenmerken van de moeder, zoals IQ ook een rol spelen in het

verschil in ontwikkeling tussen borstgevoede en flesgevoede kinderen. De verschillen tussen borstgevoede en flesgevoede kinderen zijn bijzonder subtiel en niet te detecteren op individueel niveau. Dat betekent niet dat de verschillen onbelangrijk zijn, omdat op groepsniveau wel degelijk kleine effecten per individu bij elkaar opgeteld veel effecten kunnen hebben op het welzijn van de groep in zijn geheel. In ons onderzoek kwam naar voren dat op de leeftijd van 3 maanden de borstgevoede kinderen een betere kwaliteit van de spontane motoriek lieten zien in vergelijking met kinderen die flesvoeding kregen. Nadere inspectie van de gegevens van de borstgevoede kinderen liet zien dat het gunstige effect op de spontane motoriek alleen optrad wanneer de kinderen meer dan 6 weken alleen maar borstvoeding kregen. De kwaliteit van de bewegingen van kinderen die minder dan 7 weken borstvoeding hadden gekregen was gelijk aan die van de kinderen die standaard flesvoeding kregen. In onze onderzoeksgroep waren er maar heel weinig moeders die maandenlang louter borstvoeding gaven; de meeste moeders die meer dan 6 weken uitsluitend borstvoeding gaven gingen hiermee ongeveer in totaal 9 weken mee door. Op de leeftijd van 18 maanden waren de verschillen in de ontwikkeling tussen de borstgevoede en flesgevoede kinderen niet meer aanwezig. Samenvattend, blijkt er uit ons onderzoek dat het geven van borstvoeding een gunstig effect heeft op de hersenontwikkeling van het kind. De effecten zijn door ons vastgesteld op de leeftijd van 3 maanden, echter niet op 18 maanden.

De relatie tussen de vetzuursamenstelling van de navelstreng bij de geboorte en de ontwikkeling van het kind

Voor de aanvoer van voedingsstoffen is de foetus afhankelijk van de moeder. Vetzuren worden naar het kind getransporteerd via de moederkoek en de navelstreng. De aanvoer van vetzuren wordt door allerlei factoren van de moeder bepaald waaronder het voedingspatroon van de moeder. Wij hebben van ruim 300 kinderen de wanden van de navelstrengbloedvaten kunnen onderzoeken waardoor geanalyseerd kon worden welke vetzuren voor de geboorte al aanwezig waren. Op de leeftijd van 3 maanden maten wij de verschillen in navelstrengvetzuren tussen kinderen met normale en licht afwijkende spontane bewegingen. Daaruit kwam naar voren dat kinderen die licht afwijkende bewegingen vertoonden minder arachidonzuur en essentiële vetzuren hadden in hun afvoerende navelstrengvaten dan kinderen met normale spontane bewegingen. De kinderen met minder visvetzuren (DHA) in de navelstrengvaten bewogen op de leeftijd van 18 maanden gemiddeld ietsje minder soepel en struikelden wat vaker over speelgoed op de grond. Het verband kon niet alleen door toeval bepaald zijn, maar was niet heel sterk.
Onverwacht bleken de zogenaamde *trans*vetzuren in de aanvoerende navelstreng een negatief effect te hebben op de motoriek van de kinderen op 18 maanden. Dit verband kon ook niet door het toeval bepaald zijn en was vrij sterk. Hoe meer *trans*vetzuren in de navelstreng voorkwamen des te minder efficiënt was de motoriek van het kind op 18 maanden. Of dit een oorzakelijk verband is kon echter niet met zekerheid worden aangetoond met de huidige opzet van het onderzoek. We toonden alleen een associatie aan. *Trans*vetzuren kunnen alleen via de voeding van de moeder via de navelstreng naar de foetus aangevoerd zijn. *Trans*vetzuren komen van nature weinig voor en ontstaan bij het verhitten (hydrogeneren) van vloeibare oliën en lijken nog het meeste op verzadigde vetzuren met een klein knikje erin. De voedingsindustrie verwerkt veel *trans*vetzuren in snacks, kant-en-klare maaltijden, koekjes en in het verleden in de margarine om de houdbaarheid en levensduur te verbeteren. Toen jaren geleden bekend werd dat *trans*vetzuren slecht voor hart- en bloedvaten waren werden er aanzienlijk minder

*trans*vetzuren in ons voedsel verwerkt, maar desondanks is de huidige inname van *trans*vetzuren nog behoorlijk (1,1 % van alle energie die we binnen krijgen). Dit is toe te schrijven aan de toename van het eten van meer industrieel geproduceerde banketproducten en kant-en-klaar maaltijden. Het werkingsmechanisme van de negatieve uitwerking van *trans*vetzuren is nog niet goed bekend. Wel is bekend dat de *trans*vetzuren de aanmaak van meervoudig onverzadigde vetzuren verhinderen en dat ze bij dieren in hoge hoeveelheden gedragsveranderingen kunnen veroorzaken. De praktische consequentie van ons onderzoek is het advies om tijdens de zwangerschap producten met veel *trans*vetzuren te vermijden.

Conclusies

- De toevoeging van lange keten meervoudig onverzadigde vetzuren aan de flesvoeding voor de duur van twee maanden heeft een gunstig effect op de kwaliteit van de spontane motoriek van pasgeborenen op de leeftijd van 3 maanden;
- Het louter geven van meer dan 6 weken borstvoeding heeft een gunstig effect op de kwaliteit van de spontane motoriek op de leeftijd van 3 maanden in vergelijking met pasgeborenen die minder dan 7 weken borstvoeding of gewone flesvoeding hadden gekregen;
- De kinderen die borstvoeding, gewone flesvoeding of flesvoeding met meervoudig onverzadigde vetzuren kregen, hebben een vergelijkbare ontwikkeling op de leeftijd van 18 maanden;

- Pasgeborenen die licht afwijkende bewegingen vertoonden op de leeftijd van 3 maanden hadden minder essentiele vetzuren in hun afvoerende navelstrengvaten;
- De kinderen op de leeftijd van 18 maanden bewogen gemiddeld ietsje minder efficiënt, wanneer in de aanvoerende navelstrengvaten weinig visvetzuren zaten;
- Hoe meer *trans*vetzuren in de navelstreng voorkwamen des te slechter was de motoriek van het kind op 18 maanden.

Curriculum vitae
&
List of publications

8

8 Curriculum vitae & List of publications

Curriculum vitae auctoris

Hylco Bouwstra was born on Juli 30, 1980 in Heerenveen, The Netherlands. He attended secondary school (Bogerman) in Sneek and graduated in 1999. During the last years of secondary school, he became interested in science due to an enthusiastic physic teacher Buwalda. The latter encouraged students to engage in scientific contests which resulted for Hylco Bouwstra in two awards for the final report of secondary school; Van Melsenprijs (Universiteit Nijmegen) and the second prize for the Biophysics student competition in 1999 (Vereniging voor Biofysica te Amsterdam).

In 1999, he started Medical School of the University of Groningen. Concurrently he completed four additional courses philosophy at the faculty of Philosophy, University of Groningen. In 2003, he became engaged in the LCPUFA-project which resulted in the present thesis. The MD/PhD program of the 'Junior Scientific Masterclass' enabled him to work on an alternating basis on his thesis and medical internships in a period of time of four years. He received his master degree in medical sciences in 2005. Recently he extended his research to the question on the usefulness of the assessment of the general movements in a public health setting. Hylco Bouwstra will obtain his medical (MD) degree on the day when he will defend the current thesis.

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Notes